University of Alberta

Phylogeny of Parnassiinae: Comparative analysis of DNA and morphology, with implications for the classification of the subfamily (Lepidoptera: Papilionidae)

by

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Abstract

The Parnassiinae (Lepidoptera: Papilionidae) are a group of primitive swallowtail butterflies comprising 8 genera and about 70 species that are primarily distributed in the Palaearctic region. Numerous attempts have been made during the past century to infer the phylogeny of the group based on morphological characters of adults and immature stages, but the results have been conflicting and confusing. This is a comprehensive effort to resolve the phylogeny of the group, using 7 mitochondrial or nuclear genes in conjunction with 236 morphological characters. Morphological characters were mostly introduced and discussed by previous workers, and are re-examined here. The systematic conclusions of this study differ from previous work by recognizing a third tribe, Luehdorfiini, within the subfamily Parnassiinae. The phylogeography of species in several western palaearctic genera in the subfamily is examined and cases of high nucleotide divergence are interpreted as possible speciation events.

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Chapter 1: A Synopsis of current classification and characteristics of Parnassiinae

Introduction

The subfamily Parnassiinae is a group of essentially Palaearctic butterflies that is generally considered to include eight extant genera grouped in two tribes, Parnassiini (*Hypermnestra, Parnassius, Archon*) and Zerynthiini (*Sericinus, Bhutanitis, Luehdorfia, Zerynthia, Allancastria*) (Munroe, 1961; Hancock, 1983; Collins and Morris, 1985; Häuser *et al.*, 2005). A few species of the genus *Parnassius* constitute the only representatives of the subfamily in the Western Hemisphere, where they occur in the Nearctic region (Opler and Warren, 2003). The systematic position of two fossil taxa, *Thaitites ruminiana* and *Doritites bosniackii*, remains unresolved (Hancock, 1983).

Parnassiinae butterflies live in a variety of habitats, ranging from arid deserts (*Hypermnestra*) to forests (*Luehdorfia*), lowland meadows (*Zerynthia*), and high alpine habitats (*Parnassius*). They have been studied since the time of Linnaeus (1758), who named, among others, the magnificent *Parnassius apollo*. Interest in the group grew in the 18th and early 19th century when more material became available from the Far East and the Himalayas (Smart, 1976). The breathtaking beauty of *Bhutanitis* species and the variability of the genus *Parnassius* quickly made them popular among butterfly collectors who bragged about rare and unusual specimens in their collections (Salmon, 2000). At some point in the nineteenth century, these butterflies gained so much popularity that many European museums deployed expeditions to the Himalayan region solely in search of rare and undiscovered species of Parnassiinae (Talbot, 1939). Of the three parties of collectors sent in search of *Bhutanitis lidderdali* between 1868 and 1890 by the British Museum, the first was plundered by natives, the second was stricken by fever and one of its members died, and the third had a man killed by a tiger. All three returned without success, although further specimens of this species became available a few years later in the 1890s (Talbot, 1939). Today, *Parnassius* still holds a record among the genera in the subfamily for the total number of valid specific and subspecific taxonomic names. Depending on the checklist, between 38 to 47 species are recognized, and each has many subspecies and individual forms (Bryk, 1935; Collins and Morris, 1985; Weiss, 1991-2005; Häuser *et al.*, 2005).

The higher classification of the subfamily has been controversial. Some workers in the past have considered the two tribes within the subfamily Parnassiinae (Parnassiini and Zerynthiini) to be separate subfamilies (Ford, 1944a, b; Eisner, 1974; Higgins, 1975), although most recent authors discount this idea (e.g. Häuser *et al.*, 2005). Others have proposed new subfamilies (Luehdorfiinae, see Stekolnikov and Kuznetsov, 2003) or new tribes (Hypermnestriini; see Hiura, 1980). The phylogeny of the subfamily also remains unresolved at the generic level. Molecular studies carried out to date on the group have produced inconclusive results, due in part to incomplete sampling (Caterino *et al.*, 2001; Omoto *et al.*, 2004; Katoh *et al.*, 2005).

In this chapter, I provide a taxonomic synopsis of the classification and characteristics of taxa described within the subfamily Parnassiinae. Brief notes are included on their distribution, larval food plants, and other important information published on their life history or taxonomy. The purpose of this chapter is to summarize the current taxonomic understanding of the group and to facilitate locating important literature. For this synopsis I have followed the current "classical" systematic scheme for Parnassiinae that has been employed by many previous workers (e.g. Munroe, 1961; Ackery, 1975; Hancock, 1983; Häuser *et al.*, 2005), with two tribes recognized for the subfamily: Parnassiini Duponchel, 1835 and Zerynthiini Grote, 1899. I have not examined the original descriptions of all taxa listed in this chapter as it was beyond the purpose of this thesis; also there were too many logistical problems associated with obtaining hundreds of (mostly antique or obscure) manuscripts. Instead, I have made use of several previously published lists that summarize specific and infra-specific taxonomic names within the Parnassiinae (eg. Bryk, 1934-1935; Eisner, 1974; Weiss, 1991-2005; Savella, 2005) or cover particular regions or taxa (e.g. Hesselbarth *et al.,* 1995; Sabariego and Martinez, 1991). I have taken information on type localities from these reviews in order to provide some sense of distribution for each taxon. The references section therefore includes only the literature that I have personally examined. I conducted independent literature searches online and in the libraries of the University of Alberta, particularly the Cameron Science and Technology library. Zoological Record and Web of Science databases were also used extensively in online searches.

Family Papilionidae Latreille, 1802

Subfamily Parnassiinae Duponchel, 1835

Tribe Parnassiini Duponchel, 1835

1. Genus *Parnassius* Latreille, 1804

This genus is not the main focus of this thesis; therefore I confine its treatment to a general introduction. I also address the subgenera recognized within *Parnassius* (sensu Häuser *et al*., 2005).

Having received tremendous attention from both collectors and taxonomists, *Parnassius* butterflies are among the most well studied butterflies in alpine zones. The region of greatest diversity of *Parnassius* is the eastern Palaearctic, from Pakistan to central Asia and China. A few species extend as far as Europe, Japan, and into the Nearctic (Weiss, 1991, 1995). The caterpillars of *Parnassius,* like other swallowtails, possess the peculiar structure known as an osmeterium at the back of their head. They feed on various members of Crassulaceae and Papaveraceae. Isolation of *Parnassius* populations in various mountain ranges has resulted in differentiation and subsequent description of numerous subspecies, varieties and forms (e.g. Bryk, 1935).

Despite growing interest, the exact number of "species" within *Parnassius* remains disputed, as more recent checklists present conflicting numbers, ranging from 38 (UNEP-WCMC, 2006) to 47 (Weiss 1991). Many of these species, however, are morphologically very similar.

Some European countries have local regulations protecting their *Parnassius* species (*P. mnemosyne, P. phoebus* and *P. apollo*) (Collins and Morris, 1985). The well-known species, *P. apollo*, is extinct in some European countries but is relatively common in other parts of its range. It is the only species covered by CITES, despite the fact that numerous other species of *Parnassius* are in need of immediate attention (Collins and Morris, 1985). Even the more common *Parnassius* species in Asia comprise many local subspecies and distinct populations, many of which are extremely vulnerable and on the verge of extinction. Some highly endangered *Parnassius* include *P. arcticus*, *P. ariadne, P. boedromius, P. cardinal, P. felderi*, *P. loxias, P. patricius, P. simo, P. simonius,* and nearly all Tibetan species; some of these species are listed in Red Data Books for Russia, Yakutia or Tajikistan (Dinets, 2002). Many species are known from only a few specimens, and several have been rare in collection for decades, including *Parnassius autocrator* which inhabits the northern part of the Hindukush district in Afghanistan and Tajikistan (Weiss, 1991).

Eight subgenera are generally recognized within *Parnassius*, as follows (in chronological order; from Bryk, 1935; Häuser *et al.*, 2005; Savela, 2005):

- Subgenus ***Parnassius*** Latreille, 1804. Type species: *Papilio apollo* Linnaeus, 1758.
- Subgenus ***Koramius*** Moore, [1902]. Type species: *Parnassius delphius* Eversmann, 1840.
- Subgenus ***Tadumia*** Moore, [1902]. Type species: *Papilio acco* Gray, 1852.
- Subgenus ***Kailasius*** Moore, [1902]. Type species: *Parnassius charltonius* Gray, 1852.

- Subgenus ***Eukoramius*** Bryk, 1935. Type species: *Parnassius imperator* Oberthür, 1883.
- Subgenus ***Lingamius*** Bryk, 1935. Type species: *Parnassius hardwickii* Gray, 1831.
- Subgenus ***Driopa*** Korshunov, 1988. Type species: *Papilio mnemosyne* Linnaeus, 1758.
- Subgenus ***Sachaia*** Korshunov, 1988. Type species: *Parnassius tenedius* Eversmann, 1851.

All of these genus-level names, together with a few others that are generally overlooked (e.g. *Erythrodriopa* Korshunov, 1988; *Adoritis* Koçak, 1989; *Quinhaicus* Korshonov, 1990, and *Kreizbergius* Korshunov, 1990), have been synonymized with *Parnassius* (Munroe, 1961; Hesselbarth *et al.*, 1995), although they are still used to designate "species-groups" within the genus (Omoto *et al.*, 2004; Häuser *et al.*, 2005). More recent treatments are confined to grouping *Parnassius* species under a number of species-groups that may not correspond to the subgeneric classification (Weiss, 1991-2005; Tshikolovets, 1998-2003). The number of species included in each of these subgenera (or species-groups) varies in checklists (e.g. see Bryk, 1935; Häuser *et al.*, 2005). Two molecular studies have been published on the phylogeny of the genus *Parnassius* (Omoto *et al.*, 2004; Katoh *et al.*, 2005), but these fail to resolve relationships fully within *Parnassius* at subgeneric or species levels, and provide contradictory results. Many detailed books and checklists have also been published on the taxonomy of *Parnassius* (e.g. see Weiss, 1991-2005; Tshikolovets, 1998-2003); these books usually offer high quality images and color maps, and illustrate a substantial range of morphological variability for many of the species of *Parnassius*.

2. Genus *Archon* Hübner, 1822

According to Hemming (1967), *Papilio thia* Hübner, [1805-1806], originally assigned as the type species of *Archon,* is a subjective synonym of *Papilio apollinus* Herbst, 1798 because it essentially represents the same taxon at the species level. The name *Doritis* (auct. nec Fabricius, 1807) has been incorrectly considered for a long time to have *Papilio apollinus* Herbst, 1798 as its type species, but this is invalid because it is a junior objective synonym of *Parnassius* Latreille, 1804 (Hemming, 1967). This confusion is due to the fact that Hübner (1819) used the name *Doritis* for *Papilio thia*, which was followed uncertainly by various authors until in 1872 Crotch even selected *Papilio apollinus* Herbst as the type species of *Doritis* (Hemming, 1967). The name *Dorarchon* was proposed by Rothschild much later in 1918, and is a junior synonym of *Archon* (Hesselbarth *et al.*, 1995).

The genus *Archon* has two morphologically similar species, both of which feed on *Aristolochia.* The two species, which are distributed in the Middle East to Turkey and Eastern Europe, were distinguished only recently, based on differences in life stages and genitalia (De Freina, 1985).

2.1) *Archon apollinus* (Herbst, 1798)

Geographical distribution: *Archon apollinus* is distributed in Bulgaria, Greece, Turkey, Iraq, Syria, Israel and Palestine, with roughly six subspecies (Hesselbarth *et* *al.*, 1995). More specific localities are compiled in books on butterflies of Bulgaria (Abadjiev, 1992), Turkey (Hesselbarth *et al.*, 1995), Greece (Pamperis, 1997), Lebanon (Larsen, 1974) and Israel (Benyamini, 2002). It is protected by law in Greece (Pamparis, 1997).

Literature review: The life history of *A. apollinus* is discussed and illustrated at length by Higgins (1975), De Freina (1985), Carbonell (1991), Hensle (1993), Nardelli (1993a), Hesselbarth *et al.* (1995), and Lõbel *et al.* (1996). Genitalia of *A. apollinus* have been illustrated by Higgins (1975). De Freina (1985) and Hesselbarth *et al.* (1995) present discussions on the taxonomy and subspecies of *A. apollinus*, synonymizing most of the subspecies described for *A. apollinus* with the nominal subspecies, and distinguishing *A. apollinaris* as a separate species. Köstler and Abajiev (1998) discuss the origin of the Greek population of *A. apollinus* and conclude that it was introduced from Turkey during World War I.

Larval food plant: Larvae of *A. apollinus* feed on species of *Aristolochia* (Aristolochiaceae). At least 11 species of *Aristolochia* are recorded, including *A. hastate, A. paecilantha* [= *A. scarabidula*] (Larsen, 1974), *A. parvifolia*, *A. clematis* (Bryk, 1934), *A. bodame* (Buresch, 1915; Koçak, 1977, 1982), *A. hirta*, *A. bottae, A. auricularia* (Koçak, 1982), *A. rotunda*, *A. semprevirens*, and *A. billardieri* (De Freina, 1985).

Subspecies: The proposed reclassification of subspecies of *A. apollinus* by De Freina (1985) has generally been accepted (see Hesselbarth *et al.*, 1995). Based on characters primarily from genitalia as well as life history, De Freina synonymized nearly all known subspecific names for Turkish and Greek populations proposed before 1985 with the nominal *A. apollinus* (Type locality: Izmir, Turkey), some of which include *A. a. amasina* Staudinger, 1901 (Type locality: Amasya, N. Turkey), *A. a. thracica* Buresch, 1915 (Type locality: Kuru Dagi, Turkey), *A. a. armeniaca* Sheljuzhko, 1925 (Type locality: Akbunaz, NE. Turkey), *A. a. wyatti* Koçak, 1976 (Type locality: Isparta, SW Turkey), *A. a. wageneri* Koçak, 1976 (Type locality: Marash, S. Turkey), *A. a. annii* Ondrias *et al.*, 1979 (Type locality: Greece), *A. a. bellargus* Staudinger, 1892 (Type locality: Hatay, Turkey), and *A. a. lichyi* Carbonell and Brevignon, 1983 (Type locality: Aksaray-Tarsus, S. Turkey). In Turkey, beside the nominal subspecies, two other subspecies, namely *A. a. forsteri* Koçak, 1977 (Type locality: Kastamonu, Turkey) and *A. a. nikodemusi* Stüning and Wagener, 1989 (Type locality: Ambarli, Istanbul, Turkey), are recognized as valid subspecies (Hesselbarth *et al.*, 1995).

2.2) ***Archon apollinaris*** (Staudinger, [1892])

Geographical distribution: *A. apollinaris* is known from southeast Turkey, west and northwest Iran, and northern Iraq. For localities see Hesselbarth *et al.* (1995) and Nazari (2003).

Literature review: The life history of *A. apollinaris* has been studied by De Freina (1985), Carbonell (1991) and Hesselbarth *et al.* (1995). De Freina (1985) and Carbonell (1991) also discuss the taxonomy of *A. apollinaris* in detail and provide characters differentiating it from *A. apollinus*.

Larval food plant: Like *A. apollinus,* this species feeds on *Aristolochia* (Aristolochiaceae). Host species recorded so far are *A. maurorum* (Ackery, 1975), *A. bottae* (Koçak, 1982), *A. olivieri* and *A. paecilanthea* (Hesselbarth *et al.,* 1995).

Subspecies: In addition to the nominal *A. a. apollinaris* Staudinger, [1892] (Type locality: Goman Otti; NE. Turkey), a recently discovered population, *A. a. bostanchii* De Freina and Naderi, 2003 (Type locality: Pol-e-Dokhtar, Lorestna, Iran), is the only population to have received a formal subspecific name.

3. Genus *Hypermnestra* Ménétriés, 1846

According to Hemming (1967), Ménétriés proposed the name *Hypermnestra* in 1846 as a replacement name for *Ismene* Nickerl, 1846, which is an invalid junior homonym of *Ismene* Savigny, 1816 and *Ismene* Swainson, 1820. However, the introduction of this name by Ménétriés was overlooked for a long time, and *Hypermnestra* was attributed either to Heydenreich 1851 or to Westwood, [1852] (Hemming, 1967).

The genus *Hypermnestra* is monotypic and its only species, *H. helios*, flies in the lowlands of central Asia.

3.1) *Hypermnestra helios* (Nickerl, 1846)

Geographical distribution: *H. helios has a* narrow range in Iran, Afghanistan, Pakistan, Turkmenistan, Kirghizstan, Tajikstan, and Uzbekistan (Tshikolovets, 1998, 2000, 2003). It is locally common in desert habitats (Nazari, 2003).

Literature review: The life history of *Hypermnestra helios* was studied by Le Cerf (1913) and Igarashi (1984), who gave detailed accounts of every life stage. Recent publications on butterflies of Russia and central Asia (eg. Tuzov *et al.*, 1997, Tshikolovets, 1998-2003) also treat *H. helios*. Some other particularly interesting studies include Müller (1976) who studied the microscopic properties of wing scales in *Hypermnestra* and *Parnassius*, and emphasized the importance of these characters in taxonomy. As well, Kuznetsov and Stekolnikov (1996) report similarities in genitaliac musculature between *Hypermnestra* and *Papilio machaon* and discuss the phylogenetic significance of their findings.

Larval food plant: Several species within the genus *Zygophyllum* (Zygophyllaceae) are known as food plants of *H. helios*; these include *Z. atriplicoides*, *Z. gontsharovi*, *Z. portulacoides*, *Z. fabago*, *Z. turcomanicum*, Z. *oxianum*, and Z. *macrophyllum* (Tshikolovets, 1998). *Halimiphyllum*, often treated as a synonym of *Zygophyllum*, has also been reported as a foodplant for *H. helios* (Kreuzberg, 1984).

Subspecies: Several subspecific names are available for populations of *H. helios* throughout its range, but the validity of most of these has been questioned (Tshikolovets, 1998; Nazari, 2003). The nominal subspecies, *H. helios helios* (Nickerl, 1846) (Type locality: SW Kazakhstan) is distributed throughout central Asia (Kirghizstan, SW. Kazakhstan, Turkmenistan, and Tadjikistan) (Tshikolovets, 1998). Populations from southern Tajikstan and Uzbekistan have received the name *H. helios maxima* Grum-Grshimailo, 1890 (Type locality: Uzbekistan) mainly due to their larger wingspan (Bryk, 1935). Populations from northern Iran have been called *H. helios hyrcana* Sheljuzhko, 1956 (=ssp. *persica* Neuburger, 1900 [preoccupied;

see Sheljuzhko, 1956]) (Type locality: northern Iran), while populations in southern Iran are known as *H. helios bushirica* Bang-Haas, 1938 (Type locality: southern Iran) (Sheljuzhko, 1956; Bang-Haas, 1938). Populations from Afghanistan are named *H. helios ariana* Wyatt, 1961 (Type locality: Bamian, Afghanistan) (Wyatt, 1961) and those from Pakistan are called *H. helios balucha* Moore, 1895 (Type locality: Baluchistan, Pakistan) (Moore, 1895).

Family Papilionidae Latreille, 1802

Subfamily Parnassiinae Duponchel, 1835

Tribe Zerynthiini Grote, 1835

4. Genus *Zerynthia* Ochsenheimer, 1816

Although the name *Parnalius* Rafinesque, 1815 predated *Zerynthia* Ochsenheimer, 1816, it was proposed for suppression to conserve the name *Zerynthia*, by Kudrna and Ackery (1977) as well as Riley and Higgins (1977). The ICZN granted these proposals and *Zerynthia* Ochsenheimer, 1816, has been conserved by the suppresion of *Parnalius* Rafinesque, 1815 for the Law of Priority and not for the Law of Homonymy (ICZN, 1979: 102).

Zerynthia is a well studied genus with two readily recognized species, *Z. rumina* and *Z. polyxena,* distributed in southern and eastern Europe and northern Africa (Higgins and Riley, 1970). Both species have received attention from generations of European lepidopterists, and consequently, their life histories are well known. There are at least one or two available scientific names for nearly every population (Manley and Allcard, 1970). *Zerynthia polyxena* alone, for example, has more than 32 valid subspecific names (Nardelli and Hirschfeld, 2002). Larvae of both species feed on *Aristolochia,* and their life history and ecology are also well known. Ford (1944a, 1944b) and Eisner (1974) proposed several morphological characters that can be useful in phylogenetic studies on *Zerynthia* and related genera. Intra- and interspecific hybridization between the two species has also been investigated by Descimon and Michael (1989) and Lux (1990).

4. 1) *Zerynthia polyxena* (Denis and Schiffermüller, 1775)

Distribution: This species has a wide distribution in Europe. It is recorded from the Czech Republic, Slovakia, France, Italy, Switzerland (?), Moldova, Poland, Ukraine, Austria, Slovenia, Hungary, Romania, Croatia, Bosnia-Herzegovina, Serbia, Montenegro, Kosovo, Macedonia, Albania (?), Bulgaria, and Greece (Higgins and Riley, 1970). Its range also extends into the western Caucasus, Turkey, south Urals, Kazakhstan, and east to southwest Russia (Lower Volga region) (Kudrna, 2002).

Literature review: The life history of this species has been discussed and illustrated by Higgins (1975), Acquier (1981), Pamperis 1997 (Greece), and Hesselbarth *et al* (1995). Hemming (1967) gave a detailed explanation on the nomenclatorial history of *Z. polyxena* and how this name was finally established as the valid name. Genitalia are illustrated by Higgins (1975). Coutsis (1989) pointed out variation in valvae among some European populations. Sijaric (1989) studied wing pattern variability within populations in Yugoslavia, and Lux (1990) conducted a similar study on variation within a single subspecies, *Z. polyxena creusa*, in France. Hesselbarth *et al.* (1995) have provided a historical summary of research on *Z. polyxena*.

Larval food plant: Several species of *Aristolochia* are recorded as the larval food plant of *Z. polyxena*, including *A. pallida, A. pontica, A. pistolochia, A. rotunda, A. clematitis,* and *A. sicula* (Bryk, 1934; Higgins and Riley, 1970; Ackery, 1975; Acquier, 1981; Hesslebarth *et al.*, 1995).

Subspecies: Thirty-one subspecies are considered valid today, and are listed chronologically below (from Nardelli and Hirschfeld, 2002). All synonymy and type localities are after Nardelli and Hirschfeld, 2002.

1- *Z. polyxena polyxena* (Denis and Schiffermüller, 1775) (Type locality: "Wien").

2- *Z. polyxena cassandra* (Geyer, 1828) (Type locality: France, Alpes Maritimes) (= *creusa* Meigen, 1829; Type locality: Central Italy).

3- *Z. polyxena demnosia* Freyer, 1833 (Type locality: Yugoslavia, Dalmatia, Trieste, Fiume).

4- *Z. polyxena polymnia* Millière, 1880 (Type locality: Greece, Euboea) (= *thusnelda* Fruhstorfer, 1908; Type locality: Greece, Thessalia).

5- *Z. polyxena latiaris* Stichel, 1907 (Type locality: Italy, Lazio).

6- *Z. polyxena gracilis* Schultz, 1908 (Type locality: Turkey, Bitinia, Bursa) (= *macedonia* Eisner, 1974; Type locality: Macedonia, Skopje).

7- *Z. polyxena reverdinii* Fruhstorfer, 1908 (Type locality: Italy, Liguria, Rapllo).

8- *Z. polyxena thesto* Fruhstorfer, 1908 (Type locality: Russia, Saratow).

9- *Z. polyxena latevittata* Verity, 1919 (Type locality: Sicily) (= *vipsania* Hemming, 1941; Type locality: Sicily).

10- *Z. polyxena nemorensis* Verity, 1919 (Type locality: Italy, Toscana, Forte dei Martini).

11- *Z. polyxena albanica* Riemel, 1927 (Type locality: Albania, Tirana).

12- *Z. polyxena aemiliae* Rocci, 1929 (Type locality: Italy, Emilia Romagna).

13- *Z. polyxena padana* Rocci, 1929 (Type locality: unknown).

14- *Z. polyxena taygetana* Rosen, 1929 (Type locality: Greece, Taygetos).

15- *Z. polyxena linnea* (Bryk, 1932) (Type locality: Italy, Elba).

16- *Z. polyxena petrii* (Bryk, 1932) (Type locality: Russia, Cherson, Berislav).

17- *Z. polyxena cassandra-clara* Verity, 1947 (Type locality: Croatia, Zagabria).

18- *Z. polyxena microcreusa* Verity, 1947 (Type locality: France, Roquebrune).

19- *Z. polyxena deminuta* Verity, 1947 (Type locality: France, Nice, St. Bernabè).

20- *Z. polyxena tristis* de Lattin, 1950 (Type locality: Turkey, Aydos Dagi).

21- *Z. polyxena bryki* Eisner, 1954 (Type locality: border of Montenegro and Herzegovina).

22- *Z. polyxena silana* Storace, 1962 (Type locality: Italy, Calabria, Sila Piccola).

23- *Z. polyxena bosniensis* Eisner, 1974 (Type locality: Bosnia, Dol. Tuzla).

24- *Z. polyxena idaensis* Eisner, 1974 (Type locality: Crete).

25- *Z. polyxena nigra* Sijaric, 1989 (Type locality: Bosnia-Hercegowina, Sarajevo).

26- *Z. polyxena sontae* Sijaric, 1989 (Type locality: Serbia, Backa).

27- *Z. polyxena carmenae* Sabariego and Martinez, 1991 (Type locality: Kalofer, Bulgaria).

28- *Z. polyxena decastroi* Sala and Bollino, 1992 (Type locality: Italy, Prealpi Venete) (= *aegidii* Nardelli, 1993; Type locality: Italy, Prealpi Venete).

29- *Z. polyxena michaelis* Nardelli, 1993 (Type locality: Italy, Apulia).

30- *Z. polyxena patrizii* Nardelli, 1993 (Type locality: Italy, Calabria, Costa Ionica).

31- *Z. polyxena caucasiae* Nardelli and Hirschfeld, 2002 (Type locality: Black Sea, Sothsi).

4.2) *Zerynthia rumina* (Linnaeus, 1758)

Distribution: *Z. rumina* is distributed in southern France, Spain, Portugal, Morocco, Algeria and Tunisia (Higgins and Riley, 1970).

Literature review: The life stages of *Z. rumina* are discussed by Nardelli (1993a; European *Z. rumina*) as well as Tarrier *et al.* (1994) and Binagot and Lartigue (1998) (African subspecies *africana* and *tarrieri*). Genitalia have been illustrated by Higgins (1975). For the classification of Spanish populations, see Sabariego and Martinez (1991). One form, f. *honratti* (Type locality: France), has been protected by law in France (Collins and Morris, 1985; Bernardi, 1999).

Larval food plant: The larvae feed on *Aristolochia pallida, A. baetica, A. longa, A. fontanesi, A. rotunda, A. pistolochia*, and *A. clematitis* (Bryk, 1934; Ackery, 1975; Acquier, 1981; Olivares Villegas *et al.,* 1991; Tennent, 1996; Binagot and Lartigue 1998).

Subspecies: The nominal subspecies, *Z. rumina rumina* (Type locality: S. Europe) is distributed is Spain, southern France, and Portugal (Higgins and Riley, 1970). In addition to *Z. r. australis* Esper, 1780 (Type locality: S. France) and *Z. r. lusitanica* Bryk, 1932 (Type locality: Portugal), other European subspecies are confined to Spain (Sabariego and Martinez, 1991); these include *Z. r. castiliana* Rühl, 1892 (Type locality: Kastilia: S. Ildelfonso, Albarracia, Spain), *Z. r. petheri* Romei, 1927 (Type locality: Sierra Nevada and Malaga, Spain); *Z. r. catalonica* De Sagarra, 1930 (Type locality: Catalonia, Spain), *Z. r. cantabricae* Gomez-Bustillo, 1971 (Type locality: Puerto de Pozazal, Spain), *Z. r. isabelae* Sabariego and Huertas, 1976 (Type locality: Huelva prov., Spain), and *Z. r. transcastiliana* Sabariego, 1977 (Type locality: Spain).

locality: Huelva prov., Spain), and Z. r. *transcastiliana* Sabariego, 1977 (Type locality: Spain).

The majority of the African populations belong to *Z. r. africana* (Stichel, 1907) (Type locality: Algeria, Morocco) (=ssp. *mauretanica* Schultz, 1908; Type locality: north Africa) (Tennent, 1996), although the populations from Anti-Atlas Mountains in north Africa have been recently described as *Z. r. tarrieri* Binagot and Lartigue, 1998 (Type locality: Morocco) (Binagot and Lartigue, 1998).

4. Genus *Allancastria* Bryk, 1934

The name *Allancastria* first appeared in print in 1932 in a paper by Bryk (Hemming, 1967). It has frequently been considered a subgenus of *Zerynthia* Ochsenheimer, 1816 (e.g. Hesselbarth *et al.*, 1995), and the species belonging to it have previously been classified under *Zerynthia, Thais* Fabricius, 1807, or *Parnalius* Rafinesque, 1815 (see Ackery, 1975; Hesselbarth *et al.*, 1995). According to Hemming (1967), the name *Thais* Fabricius, 1807 is invalid, as it is a junior homonym of *Thais* Röding, 1798. Ackery (1975: 90, 91) treated *Allancastria* as a junior subjective sysnonym of *Parnalius* Rafinesque, 1815, under which he included *P. cerisyi, P. rumina* and *P. polyxena,* and indicated that if *Allancastria* has to be recognized as a genus "there is equal justification for raising the status of the species groups of *Parnassius* to genera". The priority of the name *Parnalius* Rafinesque, 1815 was later suppressed by the ICZN in favor of *Zerynthia* Ochsenheimer, 1816 (ICZN opinion 1134, 1979).

Hemming (1967: 37) believed that the name *Allancastria* was invalid, since "Bryk (1932) provided no generic diagnosis and designated no type species, both essential requirements for a generic name published after the close of 1930" (Article 13, ICZN). However, according to Cowan (1970: 41), Bryk actually designated *Thais cerisyi* Godart, [1824] as the type species in 1934; and therefore the name *Allancastria* should be considered valid and available.

The genus *Allancastria,* including the only species that was then known, *Allancastria cerisy*, was first separated from *Zerynthia* by Bryk in 1934. The monotypy of *Allancastria* held for a long time, and many local forms and subspecies were described for *A. cerisyi*, some of which were later elevated to species rank. According to Häuser *et al.* (2005), the genus today consists of five species: *Allancastria cerisyi* (Godart, 1824); *A. deyrollei* Oberthür, 1869; *A. cretica* (Rebel, 1904); *A. caucasica* (Lederer, 1864); and *A. louristana* (Le Cerf, 1908). They have a western Palaearctic distribution and their larvae feed on various species of *Aristolochia* (Aristolochiaceae).

5.1) *Allancastria cerisyi* (Godart, 1824)

Distribution: Albania, Bulgaria, Cyprus, Greece, Macedonia, Romania, Serbia, Turkey, Syria, Jordan, Lebanon, and Palestine (Hesselbarth *et al.,* 1995). Older records from the Caucasus Mountains, Iraq and Iran likely belong to *A. caucasica* or *A. deyrollei* (Nazari, 2003).

Literature review: The life history of *A. cerisyi* has been extensively studied and illustrated (Igarashi, 1984; Hensle, 1993 [Turkey]; Pamparis, 1997 [Greece]; von Stetten, 2001 [Cyprus]; Hürter, 2001 [Rhodes Island and Turkey]). Higgins (1975) provided descriptions and drawings of genitalia for a specimen from Bulgaria. Crossbreeding experiments have been conducted between subspecies of *A. cerisyi* in the Balkan region (Sala and Bollino 1994) as well as between *A. cerisyi* and *A. cretica* (Hürter, 2001).

Larval food plant: A number of *Aristolochia* species (Aristolochiaceae) have been recorded as larval food plants for *Allancastria cerisyi*. These are: *A. clematitis* and *A. macedonica* in Balkan and Macedonia, *A. parvifolia* in Aegean and western Turkey, *A. guichardii* in the Island of Rhodes, *A. semprevivum* in Palestine, Syria and Lebanon; *A. auricularia, A. brevilabris, A. billardieri, A. bodamae, A. cilicica, A. hirta* and *A. pontica* in Turkey (Sala and Bollino, 1994; Hesselbarth *et al.*, 1995; Carbonell, 1996b; von Stetten, 2001). The larva also tolerates *A. longa, A. pistolochia,* and *A. rotunda* under laboratory conditions (Carbonell, 1996b; von Stetten, 2001).

Subspecies: Hesselbarth *et al.* (1995) have synonymized the available names for many of the Turkish and Greek populations of *A. cerisyi* with the nominal subspecies, *A. cerisyi cerisyi* (Type locality: W of Izmir, Turkey). Some of these synonyms include *A. c. martini* Fruhstorfer, 1906 (Type locality: Rhodos Island), *A. c. speciosa* Stichel, 1907 (Type locality: Syria; Brussa, Turkey), *A. c. cypria* Stichel, 1907 (Type locality: Cyprus), *A. c. mysiensis* Eisner and Wagener, 1974 (Type locality: Turkey), *A. c. koçaki* Kuhna, 1977 (Type locality: NW Turkey), *A. c. goeksui* Kuhna, 1977

(Type locality: Turkey), and *A. c. sami* Schmidt, 1989 (Type locality: Samos, Greece). Sala and Bollino (1997) retain the taxon *A. c. martini* as a valid subspecies. The Balkan populations have received several reviews (e.g. Sijaric and Mihljevic, 1972; Sala and Bollino, 1994) and many subspecific names have been proposed, including *A. c. ferdinandi* Stichel, 1907 (Type locality: Bulgaria), *A. c. mihljevici* Sijaric, 1990 (Type locality: Bosnia-Hercegovina), and *A. c. dalmacijae* Sala and Bollino, 1994 (Type locality: Makarska, Dalmatia, Croatia). The taxon *A. c. huberi* Sala and Bollino, 1994 (Type locality: Greece, Florina) is also often regarded as a valid subspecies in Greece (Pamparis, 1997).

5.2) *Allancastria caucasica* (Lederer, 1864)

Geographical distribution: This species is distributed from the Black Sea and southern Russia (southern Caucasus Mountains) to Georgia and northeast Turkey (Hesselbarth *et al.*, 1995).

Literature review: The life history and ecology of *A. caucasica* has been studied in detail by Hensle (1993), Nardelli (1993a, 1993c), Hesselbarth *et al.* (1995), and von Stetten (2002). Described originally as a subspecies of *A. cerisyi*, *A. caucasica* was first given species status by Kuhna (1977). Hesselbarth *et al.* (1995) provided synonymies for *A. caucasica* (Type locality: Kutaisi, Georgia), including *A.c. tkatschukovi* Sheljuzhko, 1927 (Type locality: Sotshi, Caucasus), *A.c. cachetica*

Sheljuzhko, 1927 (Type locality: Lagodechi, SE Caucasus), and *A.c. abanti* Koçak, 1975 (Type locality: Abant Golu, Bolu prov., Turkey).

Larval food plant: Larvae feed on *Aristolochia pontica, A. pallida*, and *A. iberica* (Carbonell 1997; Hesselbarth *et al.* 1995). In captivity, larvae also tolerate *A. pistolochia* and *A. rotunda* (Nardelli, 1993a, 1993c; Carbonell, 1996b).

5.3) *Allancastria deyrollei* Oberthür, 1869

Distribution: *Allancastria deyrollei* is distributed from western Iran to Turkey, Syria, northwestern Iraq, Lebanon, Jordan, and Israel (Hesselbarth *et al.* 1995; Nazari 2003).

Literature review: First given species rank by Larsen (1976), the life history of *Allancastria deyrollei* has been studied extensively (De Freina, 1979, 1987; Nardelli, 1993a; Hesselbarth *et al.*, 1995; Hensle, 1993). No subspecies are recognized; the names *A. d. eisneri* Bernandi, 1971 (Type locality: Aintab, Turkey) and *A. d. lycaoniae* Eisner and Wagner, 1974 (Type locality: Aksehir, Konya, Turkey) have been synonymized with nominal *A. d. deyrollei* (Type locality: Pontus, Trabzon, Turkey) (Hesselbarth *et al.*, 1995). Larsen (1973) compared the structure of genitalia in *A. deyrollei* with that of *A. cerisyi*, and De Freina (1979) provided further characters distinguishing *A. cerisyi* from *A. deyrollei*. Carbonell and Karbalaye (1998) mapped the records of *A. deyrollei* (and a few other *Aristolochia*-feeder species) in relation to the distribution of *Aristolochia* in Iran.

Larval food plant: Larvae of *Allancastria deyrollei* feed on *Aristolochia maurorum, A. bottae* and *A. paecilantha* (DeFreina, 1979; Carbonell, 1996b; Carbonell and

Karbalaye, 1998). *A. clematitis* has also been recorded as a larval food plant (Nardelli, 1993a).

5.4) *Allancastria cretica* (Rebel, 1904)

Geographical distribution: *Allancastria cretica* is endemic to the island of Kriti (Crete) (Type locality) in Greece (Carbonell, 1996b; Pamparis, 1997).

Literature review: First given species rank by Koçak (1989), the life history of *A. cretica* has been studied by Carbonell (1996b), Dennis (1996), Pamparis (1997) and Hürter (2001). No subspecies are recognized. Higgins (1975) and Sala and Bollino (1997) studied the structure of genitalia of *A. cretica* in comparison to other species of *Allancastria* (Higgins, 1975). Carbonell (1996b) considered *cretica* a valid species and studied its taxonomic relationship with other species of *Allancastria*. Hürter (2001) described inter-specific hybrids with *A. cerisyi*.

Larval food plant: Two species of *Aristolochia* are used by larvae in Crete: *Aristolochia cretica* and *Aristolochia semprevirens* (Carbonell, 1996b; Dennis, 1996). The larva tolerates two other species in captivity: *A. pistolochia* and *A. longa* (Carbonell, 1996b).

5.5) *Allancastria louristana* (Le Cerf, 1908)

Geographical distribution: The range of *A. louristana* is restricted to western Iran.

Literature review: The life history of *A. louristana* (Type locality: Louristan, "Perse") has been studied by Carbonell (1996a) and Carbonell and Karbalaye (1998).

Blom and Eisner (1979) described *A. l. boyrahmadensis* (Type locality: Yasuj, Iran) based on differences in a few morphological characters; this name was later synonymized with the nominal population (Nazari, 2003). Carbonell and Karbalaye (1998) map the records of *A. louristana* (and some other *Aristolochia*-feeding species), in relation to the distribution of *Aristolochia* in Iran.

Larval food plant: Only *Aristolochia olivieri* is recorded as a larval food plant of *A. louristana* (Carbonell, 1996a, 1996b).

6. Genus ***Bhutanitis*** Atkinson, 1873

Blanchard (1871) described the species *thaidiana* under new genus *Armandia*. The generic name *Armandia* Blanchard, 1871 is invalid as it is a junior homonym of *Armandia* Filippi, 1862 (Hemming, 1967). Atkinson (1873) described the species *lidderdalii* under a new genus *Bhutanitis*. After the description of *Armandia mansfieldi* by Riley in 1939, based on an unlabeled specimen from China, doubts were raised as to whether this new species belonged to *Bhutanitis* since it apparently shared many morphological characters with *Luehdorfia*. In 1980 Hiura described a new genus, *Yunnanopapilio* (Type species: *B. mansfieldi*). Based on genitalic characters, *Yunnanopapilo* was later synonymized with *Bhutanitis* (Saigusa and Lee, 1982). Further evidence on the life history of *B. mansfieldi* supported this decision (Igarashi, 2003). Lee (1986) uses the generic names *Yunnanitis* and *Sinonitis* for *B. mansfieldi* and *B. thaidiana* with no reference to their authorship and publications. Since these names do not seem to be indicated anywhere else, they are treated as *numina nuda* here. A recent molecular study (Zhu *et al.,* 2005) investigates the phylogenetic relationships among the species of *Bhutanitis* using sequences of *COI* mitochondrial gene, and provides evidence for synonymy of the taxon *Bhutanitis mansfieldi pulchristrata* with nominal *B. m. mansfieldi*.

Today *Bhutanitis* has four recognized species distributed from northern India and China to Thailand (Häuser *et al.*, 2005). All four species have been protected by law for a long time (Collins and Morris, 1985; Coote, 2000), but recently two of these species (*B. ludlowi* and *B. mansfieldi*) are listed by IUCN in 2004 as vulnerable or data deficient (IUCN, 2004; UNEP-WCMC, 2006).

6.1) *Bhutanitis lidderdalii* Atkinson, 1873

Geographical distribution: This species is found in northern India, northern Burma, Bhutan, west and southwest China, and northern Thailand (Chou, 1994).

Literature review: The life history of this species has been discussed in detail by Talbot (1939) and Igarashi (1989). The genitalia have been illustrated and discussed by Saigusa (1973), Saigusa and Lee (1982) and Chou (1992). Saigusa (1973) proposed a system of wing pattern character coding based on the wing pattern of *B. lidderdali*.

Larval food plant: The larvae of *B. lidderdali* feed on species of *Aristolochia* (Aristolochiaceae). Recorded host species include *A. griffithii, A. kaempferii, A. mandshuriensis, A. shimadai* and *A. debilis* (Igarashi, 1985, 1989).

Subspecies: Beside the nominal subspecies, *B. lidderdalii lidderdalii* Atkinson, 1873 (Type locality: Buxa, Bhutan), three other subspecies are recognized: *B. l. spinosa* Stichel, 1907 (Type locality: Sichuan, W. China.), *B. l. ocellatomaculata* Igarashi, 1979 (Type locality: Chiang-Mai, northern Thailand), and *B. l. nobucoae* Morita, 1997 (Type locality: N. Kachin, Myanmar) (Coote, 2000).

6.2) *Bhutanitis thaidina* (Blanchard, 1871)

Geographical distribution: Southwest China (Tibet, Nanshan, Szechwan, Yunnan, Shaanxi) (Chou, 1994).

Literature review: For information on life history, see Collins and Morris 1985 (IUCN book) and Lee (1986). Genitalia have been illustrated and discussed by Chou (1992), Saigusa and Lee (1982), and Bai and Wang (1998).

Larval food plant: Two species of *Aristolochia* are recorded as host plants of *B. thaidiana*; these are *A. moupinensis* and *A. delavayi* (Lee, 1986; Chunsheng, 2001).

Subspecies: The type locality of the nominal subspecies is not given. A recognized subspecies is *B. t. dongchuanensis* Lee, 1986 (Type locality: Dongchuan, Yunnan, China) (Lee, 1986). Chou (1992) described *B. yulongensis* (Type locality: Mt. Yulong, Yunnan, S. China) and *B. nigrilima* (Type locality: Kangding, Sichuan, S. China) as valid species, but others have listed them as subspecies or synonyms of *A. thaidiana* (see Igarashi 2003).

6.3) *Bhutanitis ludlowi* Gabriel, 1942

Bhutantis ludlowi is known only from the type series collected in the Trashiyangsi valley in northeastern Bhutan (Type locality) in 1933 and 1934 (Gabriel, 1942). Recently it has also been collected in China, southwestern Sezchuan (Coote, 2000;

Anonymous [2005]). Practically nothing is known about the life history of this species. For further information, see Collins and Morris (1985).

6.4) *Bhutanitis mansfieldi* (Riley, 1939)

Geographical distribution: *B. masfieldi* is endemic to China (Yunnan, Sichuan) (Saigusa and Lee, 1982).

Literature review: The life history has been discussed in detail by Igarashi (2003). According to Riley, who described the species in 1939, the type specimen was discovered by M.J. Mansfield among papered and unlabeled specimens from the collection of George Forrest, a British botanist who extensively collected in Yunnan, China. Riley therefore assumed that the type must have been collected in Yunnan. Morphological characters are discussed by Saigusa and Lee (1982) and Igarashi (2003), as well as Collins and Morris (1985). Hiura (1980) erected a new genus, *Yunnanopapilio* Hiura, 1980, for *B. mansfieldi*, based on some wing pattern characters as well as venation; this name was later synonymized with *Bhutanitis* by Saigusa and Lee (1982).

Chou (1992) gave species rank to *B. m. pulchristrata*, but this designation is generally not accepted. Genitalia have been illustrated by Chou (1992) and by Bai and Wang (1998).

Larval food plant: Only *Aristolochia moupinensis* has been recorded as a larval host plant in the wild (Lee, 1986; Chunsheng, 2001). In captivity, larvae also tolerate *A. griffithii*.

Subspecies: Two subspecies are recognized: *B. mansfieldi mansfieldi* (Riley, 1939) (Type locality: Yunnan, China), and *B. mansfieldi pulchristata* Saigusa and Lee, 1982 (Type locality: Sichuan, China) (Saigusa and Lee, 1982; Coote, 2000), although a recent molecular study has suggested that the name *B. m. pulchristrata* should be synonymized with nominal *B. m. mansfieldi* (Zhu *et al.*, 2005).

7. Genus *Luehdorfia* Crüger, 1878

Luehdorfia eximia Crüger, 1878, the type species originally described by Crüger (1878) and designated as the type species for the new genus *Luehdorfia* is now treated as a subjective synonym of *Thais puziloi* Erschoff, 1872 (Hemming, 1967). The original description of the genus was in the form *Lühdorfia*, but since this method of spelling is not permitted by the ICZN, it was corrected to *Luehdorfia* (Hemming 1967).

There are currently four species recognized within the genus *Luehdorfia*. They are distributed in China, Korea, Russian Far East, and Japan, and have larvae that feed on species of *Asarum, Heterotropa,* and *Asiasarum* (Aristolochiaceae) (Matsumoto, 1989; Matsumoto *et al.*, 1993; Igarashi, 2003).

Numerous studies on the distribution, wing pattern, life history, behavior, population dynamics, cross breeding, chromosomes and biochemistry of *Luehdorfia japonica* and *L. puziloi* have been published by Japanese researchers (for a detailed list of such publications, see Makita *et al.*, 2000). The relationship of the species within *Luehdorfia* has received particular attention. Takahashi (1973) and Hiura

(1978) suggested that *L. japonica* diverged from *L. puziloi inexpecta* as a result of host shift from the deciduous genus *Asiasarum* to the evergreen genus *Asarum*. Shinkawa (1991) and Watanabe (1996) suggested that *L. puziloi* colonized the Japanese Archipelago and gave rise to *L. japonica*, and that *L. chinensis* is the most basal species of the genus *Luehdorfia*. At least three phylogenetic reconstructions have been proposed for the species within *Luehdorfia*, including Kato (1998) based on genitalia characters, Makita *et al*. (2000) based on 785 bp of mtDNA (*ND5*), and Matsumura *et al.* (2005) based on *COI* and *ND5*. Results of these studies are different from those proposed by Takahashi (1973) and Hiura (1978), as well as contradicting each other.

7.1) ***Luehdorfia puziloi*** (Erschoff, 1872)

Geographical distribution: Northern China, southeast Russia, North- and South Korea, Japan (Shirozu and Hara, 1973).

Literature review: The life history of *L. puziloi* has been illustrated by Shirozu and Hara (1973) and Nardelli (1993a). Biochemical and biological aspects of the life history of *L. puziloi* have been the subject of a number of studies by Matsumoto (1984-1990), Matsumoto *et al.* (1993), and Tsubaki and Matsumoto (1998). Hybrids between *L. japonica* and *L. puziloi* have been reported (Hara and Ochiai, 1980). There has been some controversy about the authorship of the ssp. *linjiangensis*; details are reported under *L. taibai*.

Larval food plant: The larvae of *L. puziloi* feed on *Asarum sieboldii* (Chunsheng, 2001) as well as some species of *Asiasarum* (Makita *et al.*, 2000).

Subspecies: At least seven subspecies are recognized within *L. puziloi.* According to Fujioka (2003), the nominal subspecies, *L. p. puziloi* Erschoff, 1872 (Type locality: Ussuri region) is confined to southeast Russia, together with *L. p. machimuraorum* Fujioka, 2003 (Type locality: Russia: Okhotniki, upper Bikin River). According to Bryk (1934), two subspecies, *L. p. yessoensis* Rothschild, 1918 (Type locality: Hakodate Yezzo, Japan) and *L. p. inexpecta* Sheljuzhko, 1913 (Type locality: Sendai, Japan) are known from Japan, and the populations on the Korean peninsula belong to *L. p. coreana* Matsumura, 1919 (Type locality: Kaishu, Korea). In China, two subspecies are known: *L. p. linjangensis* Lee, 1982 (Type locality: China) and *L. p. lenzeni* Bryk, 1938 (Type locality unknown) (Savela, 2005).

7.2) *Luehdorfia japonica* Leech, 1889

Geographical distribution: Japan (central and western Honshu); China (Taiwan?) (Shirozu and Hara, 1973).

Literature review: Life stages of this species are illustrated in Shirozu and Hara (1973).

Besides the nominal *L. japonica* (Type locality: Japan), no other subspecies are recognized, the population from Taiwan (*L. j. formosana* Rothschild, 1918) is either an erroneous record or is extinct since this species does not occur in Taiwan today (Li, 1987). Hybrids between *L. japonica* and *L. puziloi* have also been reported (Hara and Ochiai, 1980).

Larval food plant: Many species of *Asarum* (Aristolochiaceae) have been recorded as larval foodplants of *Luehdorfia japonica*; these include: *Asarum caulescens, A.*

sieboldii, A. hexalobum, A. asperum, A. tamaense, A. curvistigma, A. asaroides, A. blumei, A. nipponicum, A. fauriei, A. takaoi, A. kurosawae, A. heterotropoides, A. megacalyx, and *A. yohikawai* (Makita *et al.*, 2000).

7.3) *Luehdorfia chinensis* Leech, 1893

Geographical distribution: Central and eastern China (Chou, 1994).

Literature review: The life history of *L. chinensis* has been studied by Cui *et al.* (1992), Yuan *et al.* (1998), and Yao *et al.* (1999). There has been some controversy about the authorship of subspecies *huashanensis*; see under *L. taibai.*

Larval food plant: Two species of *Asarum* have been recorded as food plants of *Luehdorfia chinensis*; these are *Asarum forbesii* (Makita *et al.*, 2000, Chunsheng, 2001) and *Asarum sieboldii* (Chunsheng, 2001).

Subspecies: The nominal subspecies, *L. chinensis chinensis* Leech, 1889 (Type locality: Chang-Yang, Lu Shan Mts. [KiuKiang], central China) and the recently described ssp. *leei* Chou, 1994 (=*huashanensis* Lee, 1982; see under *L. taibai* for details) (Type locality: Shaanxi, Ningshan, China) are the only known supspecies of *L. chinensis*.

7.4) *Luehdorfia taibai* Chou, 1994

Geographical distribution: China: Shaanxi (Lee, 1982).

Literature review: Nothing is known about the life history of *L. taibai.*

The name *Luehdorfia longicaudata* Lee, 1982, often used instead of *L. taibai*, is invalid. It was used first in a paper by Lee (1982: 39), who only reported the distribution and living conditions of the three *Luehdorfia* taxa in China, and suggested scientific names for them (*L. longicaudata, L. chinensis huashanensis*, and *L. puziloi lingjiangensis*) without giving any descriptions of morphological and diagnostic characters, reference to any figures, or designation of any holotypes for these taxa. This is in violation of a number of ICZN (1999) articles and recommendations, and despite attempts to conserve these names, especially by Inomata (1995), they were all subsequently treated as synonyms of other taxa by Yuan (1995). The suggested - and widely accepted – alternative name for this species is *L. taibai* Chou, 1994, with the type locality "Mt. Taibaishan, China".

Larval food plant: The larval food plant records for *L. taibai* include *Saruma henryi* (Makita *et al.,* 2000, Chunsheng, 2001), species of *Asarum,* and even *Aristolochia* spp. (Chou, 1994).

Subspecies: The nominal subspecies, *L. taibai taibai* Chou, 1994 (Type locality: Mt. Taibaishan, China) and the recently described *L. t. wangi* Zhao, 1997 (Type locality: China: Sichuan, Mt. Daba) are the only known subspecies of *L. taibai (*Zhao, 1997).

8. Genus *Sericinus* Westwood, 1851

The genus *Sericinus* (Type species by monotypy: *Papilio telamon* Donovan, 1798 = *montela* Gray, 1852) (Hemming, 1967), and its only species, *S. montela,* is distributed in the Russian Far East, Korea, China, and Japan (Ackery, 1975). Its larvae feed on

Aristolochia, and it is the only multi-voltine species in the Parnassiinae (Igarashi 2003).

8.1) ***Sericinus montela*** Gray, 1852

Geographical distribution: China, North and South Korea (Ackery, 1975), Vladivostok, Amur, Ussuri (Tuzov *et al.*, 1997). It has been recently introduced to Japan (Kumon, 2005).

Literature review: The name *Sericinus montela* Gray, 1852 was proposed as a replacement for the preoccupied *Papilio telamon* Donovan, 1798 (preoccupied by *Papilio telamon* Linnaeus, 1758). *P. telamon* Donovan,1789 was originally designated as the type species of *Sericinus* (Hemming, 1967). The life history of this species has been studied extensively by Monastyrisky and Kotlobay (1995), Li (1983), Igarashi (1984) and Nardelli (1993a). There is very little published research on other aspects of *S. montela*; Kwon and colleagues (1995) studied the hemocytic (bolld cell) differentiation in *S. montela*.

Larval food plant: The larvae of *S. montela* feed on *Aristolochia contorta* (Aristolochiaceae) in the wild, and on *A. debilis* and *A. clematitis* under lab conditions (Monastyrski and Kotlobay 1995).

Subspecies: This is a relatively variable butterfly and a number of subspecies are recognized (Bryk, 1934). The nominal subspecies, *S. montela montela* Gray, 1852 (Type locality: Shanghai; central China) flies in China (Shanghai, Peking, Lungtau, east Tien-Muschen, Mt. Paoschan [Nanking]), as well as S. Ussuri, Russia. Most other subspecies, including *S. m. absurdus* Bryk, 1913 (Type locality: S. Shantung, E.

China), *S. m. elegans* Bryk, 1913 (Type locality: China), *S. m. magnus* Fruhstorfer, 1913 (Type locality: Kiangsi, south China), *S. m. mandshuricus* Rosen, 1929 (Type locality: north Manchuria, northeast China), and *S. m. guangxiensis* Bai and Wang, 1998 (Type locality: China) are also described from and restricted to China (Bryk, 1934; Bai and Wang, 1998). Korea has three subspecies: *S. m. koreanus* Fixsen, 1887 (Type locality: Korea: Seoul, Kwangnoeng), *S. m. eisneri* Bryk, 1932 (Type locality: North Korea: vic. Sei-shisi), and *S. m. songdoi* Seok, 1933 (Type locality: Songdo, Korea) (Bryk, 1934). Subspecies *S. m. amurensis* (Staudinger, 1892) (Type locality: Ussuri: Sudschanski Rudnik, Amur merid) is recorded from Amur, Sutchansk, and Ussuri (Bryk, 1934).

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Chapter 2. Phylogeny, historical biogeography, and taxonomic ranking of Parnassiinae (Lepidotera, Papilionidae) based on morphology and seven genes

Abstract. – I tested the taxonomic utility of morphology and seven mitochondrial or nuclear genes in a phylogenetic reconstruction of the swallowtail butterflies in subfamily Parnassiinae. My data included 236 morphological characters and DNA sequences for several genes that are commonly used to infer lepidopteran relationships (*COI+COII*, *ND5*, *ND1*, *16S*, *EF-1*α, and *wg*; total 5775 bp). Nuclear genes performed best for inferring phylogenies, particularly at higher taxonomic levels, and there was substantial variation in performance among mitochondrial genes. Multiple analyses of molecular data (MP, ML and Bayesian) consistently produced a tree topology different from that obtained by morphology alone. Based on molecular evidence, sister-group relationships were confirmed between the genera *Hypermnestra* and *Parnassius*, as well as between *Archon* and *Luehdorfia*, while the monophyly of the subfamily was weakly supported. I recognize three tribes within Parnassiinae, with *Archon* and *Luehdorfia* forming the tribe Luehdorfiini Tutt, 1896 [stat. rev.]. Three fossil taxa were incorporated into a molecular clock analysis with biogeographic time constraints. Based on dispersal-vicariance (DIVA) analysis, the most recent common ancestor of Parnassiinae occurred from the Iranian Plateau and Central Asia to China. Early diversification of Parnassiinae took place at the same time that India collided into Eurasia, 65-42 million years ago.

Keywords: Phylogenetic information content, butterfly evolution, divergence time estimation, Palaearctic biogeography.

Introduction

Phylogenetic studies of insects have used DNA sequences from a multitude of gene regions, whether mitochondrial, nuclear, protein-coding or ribosomal, with the aim of finding regions that provide informative data useful for resolving phylogenies at various levels. These studies generally only assess the utility of individual gene regions or compare them to one or two others in a phylogenetic context (Simon *et al.* 1994; Brower and Desalle, 1994, 1998; Vila and Bjorklund, 2004; Danforth *et al.*, 2004, 2005; Silva-Brandão *et al.*, 2005; Wahlberg *et al.*, 2005b; Whinnett *et al.*, 2005; Wilkerson *et al.*, 2005; but see Mallarino *et al.*, 2005; Giribet and Edgecombe, 2005). The popularity of some genes, particularly those encoded by mitochondrial DNA, may primarily be due to lab tradition or the ease of their amplification, and not necessarily the phylogenetic information contained in them (Caterino *et al.*, 2000; Sperling, 2003). However, the choice of sub-optimally informative genes for a particular taxonomic level, together with incomplete sampling and missing data, can contribute to a poorly resolved phylogeny.

I examined divergence patterns of five mitochondrial and two nuclear genes in a phylogenetic analysis that included all genera and subgenera of the subfamily Parnassiinae (Papilionidae). These small swallowtail butterflies comprise eight extant genera and about 70 species with an eastern or western Palaearctic distribution pattern. The Parnassiinae have been considered the sister group of all remaining Papilionidae except for the monotypic genus *Baronia* (Hancock, 1983). *Allancastria*, *Zerynthia, Archon*, and *Hypermnestra* range from Europe to central Asia, while *Sericinus*, *Luehdorfia*, and *Bhutanitis* are distributed from Bhutan to eastern Russia and Japan (Fig. 2.1). *Parnassius* has a Holarctic distribution with the highest diversity in the Himalayas (Bryk, 1935; Weiss, 1991-2005). The larvae of most Parnassiinae genera feed on *Aristolochia* and other Aristolochiaceae, although larvae of *Hypermnestra* feed on Zygophyllaceae and those of *Parnassius* feed on Papaveraceae and Crassulaceae (Igarashi, 1984).

The subfamily Parnassiinae is generally considered to consist of two tribes: Parnasiini (including *Archon*, *Hypermnestra*, and *Parnassius*), and Zerynthiini (including *Allancastria*, *Sericinus*, *Zerynthia*, *Luehdorfia*, and *Bhutanitis*) (e.g. Ehrlich, 1958; Munroe, 1961; Ackery, 1975; Igarashi, 1984), although there has been some disagreement over whether these tribes should be regarded as separate subfamilies (Bryk, 1934; Talbot, 1939; Ford, 1944b; Chunsheng, 2001) or even families (Clench, 1955; Hemming, 1960; Eisner, 1974) (Fig. 2.2).

Lack of a well-resolved phylogeny for the Parnassiinae (Fig. 2.3), together with taxonomic uncertainties within the group, has contributed to confusion over the classification of the subfamily. Despite numerous attempts to infer the phylogeny of the group using characters from morphology (adult anatomy, wing venation and pattern, male and female genitalia, immature stages, and other ecological or biochemical characters) as well as DNA, the phylogenetic relationships within Parnassiinae remain largely unresolved. Past morphological studies have produced contradictory results (e.g. Hiura, 1980 and Hancock, 1983), and all molecular studies published so far lack representatives of some genera (Caterino *et al.*, 2001; Omoto *et al.*, 2004; Katoh *et al.*, 2005) (Fig. 2.4). Moreover, the monophyly of the subfamily itself has frequently been questioned (Häuser, 1993; Hesselbarth *et al.*, 1995; Yagi *et al.*, 1999; Caterino *et al.*, 2001; Stekolnikov and Kuznetsov, 2003).

Taxonomic uncertainties exist over the composition and ranking of most genera in the Parnassiinae. Although the genus *Parnassius* sensu lato is generally considered monophyletic (cf. Omoto *et al.*, 2004; Katoh *et al.*, 2005), the clade is often split into smaller genera or species-groups based on minor morphological differences (Bryk, 1935; Korb, 1997) that are of questionable validity (Hesselbarth *et al.*, 1995). Over a century ago, the monotypic genus *Hypermnestra* was placed in the genus *Parnassius* (e.g. Doubleday and Westwood, 1847; Gray, 1853; Moore, 1895) or *Doritis* (=*Archon*) (e.g. Herrich-Schäffer, [1856]). *Allancastria* has been treated as a synonym, or a subgenus, of *Zerynthia* (Hesselbarth *et al.*, 1995), or the two have been collectively called *Parnalius* (Ackery, 1975). Species in the genus *Bhutanitis* have received several generic names (*Yunnanopapilio, Sinonitis*, and *Bhutanitis*; Lee, 1986).

Above the genus level, correct placements of the genera *Archon, Hypermnestra* and *Luehdorfia* have been controversial (Fig. 2.2). *Archon* has sometimes been included under Zerynthiini (Eisner, 1974; Higgins, 1975) or in a separate sub-tribe within Parnassiini (Koçak, 1989). Häuser (1993) suggested a separate subfamily for *Hypermnestra* based on a number of morphological and ecological autapomorphies, a view previously expressed by Dujardin (1965), Hiura (1980), and Korshunov (1990) (as reported by Korb, 1997). A recent study of characters of the genitalia (Stekolnikov and Kuznetsov, 2003) recognized the tribe "Hypermnestriini Hiura 1980" and gave subfamily status (Luehdorfiinae Tutt, 1896) to *Luehdorfia* based on putatively primitive genitalic characters (also see Ford, 1944b). Recent findings, including the discovery of mitochondrial DNA sequence affinity between *Archon* and *Luehdorfia* (Omoto *et al.*, 2004; Katoh *et al.*, 2005) complicate the picture further. Moreover, the phylogenetic significance of two well-documented fossil species within Parnassiinae (*Thaites ruminiana* Scudder, 1875 from the lower Oligocene, Aix-en-Provence, southern France, and *Doritites bosniaskii* Rebel, 1898 from Miocene, Tuscany, Italy) has been generally overlooked, and despite suggestions by their original describers and Hancock (1983), their position in the Parnassiinae remains uncertain.

Although not generally considered to belong to Parnassiinae, the phylogenetic relationship of the genus *Baronia* to the subfamily remains intriguing. The only member of the subfamily Baroniinae, *Baronia brevicornis* is found in Mexico, and is often considered to be a "living fossil" (Collins and Morris, 1985; Tyler *et al.*, 1994; Scriber *et al.*, 1995; Eisner, 2003). The presence of many plesiomorphic traits in *Baronia* supports its position as the most primitive living swallowtail (Munroe, 1961; Hancock, 1983). The only DNA sequence study on *Baronia*, using *COI-COII* and *EF-1α* sequences, failed to resolve its position relative to the Parnassiinae (Caterino *et al.*, 2001). Here I tried to determine whether additional DNA sequence evidence would help to resolve the phylogenetic placement of *Baronia*.

This is the most comprehensive attempt to date, in terms of characters as well as sampling of genera and species, to infer the phylogeny of Parnassiinae. I re-examine morphological characters used by previous workers and combine all informative characters with 5775 bp of mitochondrial and nuclear DNA sequences to resolve several long-standing controversial issues concerning the classification of the subfamily. The purpose of this study is to: a) establish the phylogeny and classification of Parnassiinae; b) reconstruct the biogeography and evolutionary path of selected ecological characters of the Parnassiinae, and c) compare the efficacy of several widely used genes for phylogenetic reconstruction.

Materials and Methods

Taxon sampling

Sampling was comprehensive for all generic and subgeneric taxa within the traditional Parnassiinae (Table 2.1). Except for *Parnassius*, I attempted to sample every known species within the subfamily. For *Parnassius*, single species from each of the 8 major species-groups (Omoto *et al.*, 2004) were selected. No specimens of *P. hardwickii* could be obtained, and so only the sequences available on GenBank were used. Outgroups were chosen to represent major butterfly families (Hesperiidae, Lycaenidae, Nymphalidae and Pieridae). Members from all subfamilies within Papilionidae, including *Baronia brevicornis* (Baroniinae) and 3 representatives from each of the three main tribes within the Papilioninae, were selected as additional in-group taxa for all analyses. Selection was to some extent based on the availability of previously published sequences (Caterino *et al.*, 2001; Wahlberg *et al.*, 2005b). Three fossil taxa were included: *Praepapilio colorado* Durden and Rose, 1978 (Colorado, Middle Eocene), *Thaites ruminiana* Scudder, 1875 (Aix-en-Provence, Lower Oligocene), and *Doritites bosniaskii* Rebel, 1898

(Tuscany, Miocene); morphological and other data on each of these taxa were obtained using original descriptions and figures.

Dried, un-relaxed specimens were received as donations or purchased from international suppliers. Permits were obtained for all species listed under the Convention on International Trade of Endangered Species (CITES). *Bhutanitis lidderdalei* specimens were solicited by the first author from confiscated material deposited at the Canadian National Collection in Ottawa. No specimens of *Bhutanitis ludlowi* were available, and despite several attempts, some specimens (including *Luehdofia chinensis*) did not yield any usable DNA. Voucher specimens and extracted DNA samples are deposited in the E. H. Strickland Entomological Museum, University of Alberta.

Morphological characters

Morphological characters used by previous workers (Ford, 1944a, 1944b; Ehrlich, 1968; Munroe, 1961; Hiura, 1980; Saigusa and Lee, 1982; Hancock, 1983; Igarashi, 1984; Miller, 1987; De Jong *et al.*, 1996; Kato, 1998; Ackery *et al.*, 1999) were re-examined wherever possible, but in some cases taken from the literature (Appendix 1). Characters considered difficult to score (Appendix 2) or those invariant among in-groups in this study were excluded, and some new characters were proposed. Geographical distribution, larval food plant, larval gregariousness and habitat type were excluded from the analysis and reconstructed on the best-supported phylogeny (Appendix 3). In most cases, vouchers for the specimens used in DNA extractions were used in coding and checking morphological characters

(Appendix 4). Gross morphological characters were examined under a Wild-Heerbrugg dissecting microscope, and wing scales were prepared and examined with a Leitz *Laborlux S* compound microscope. Morphological character coding is presented in Appendix 5.

To facilitate reliable and consistent coding of wing pattern elements, several wing pattern models previously proposed for Parnassiinae and Papilionidae were evaluated (Eimer, 1889-1895; Verity, 1911; Hiura, 1980-1981; Smith and Vane-Wright, 2001). Wherever possible, homologies were incorporated in a basic model that included all 3 subfamilies of Papilionidae, but these codings were not extended to outgroups due to difficulties in unambiguously assigning character states. Although there were some coding errors in Hiura's (1980) analysis of the wing patterns of Parnassiinae (due to oversimplified template patterns used in his analysis that did not take into account individual variation), this system for naming bands and other wing markings was found to be the most practical, and the model used in my study is therefore a modified version of Hiura's (1980) model. All characters used by Hiura and other workers were re-analyzed, but some were discarded due to unreliability or inapplicability.

Genitalia were prepared and photographed for both males and females. In some cases where male or female specimens were not available for examination or particular structures were used up in DNA extraction (e.g. legs and thorax), character states were coded as missing data. Life history, ecology, and fossil characters were coded using the available literature. All specimens used for evaluation of morphological characters, including those used in genitalia preparations, are deposited in the E.H. Strickland Entomological Museum, University of Alberta. Specimen data are also available at http://www.biology.ualberta.ca/old_site/uasm//Vouchers/index.html.

Genes

Amplifications of mitochondrial cytochrome oxidase subunit I (*COI*)+tRNA-leucine+cytochrome oxidase subunit II (*COII*) (2310 bp), NADH-dehydrogenase subunit 5 (*ND5*) (816 bp), NADH-dehydrogenase subunit 1 (*ND1*) (472 bp), and *16S* ribosomal RNA (533 bp), as well as the nuclear protein-coding genes elongation factor 1 alpha (*EF-1α*) (1240 bp) and wingless (*wg*) (404 bp) (total 5775 bp) were obtained for any taxa and genes that had not previously been sequenced and available on GenBank. These genes were selected based on their wide phylogenetic utility in published studies on swallowtail butterflies (e.g. Aubert *et al.*, 1999; Yagi *et al.*, 1999; Caterino *et al.*, 2001; Zakharov *et al.*, 2004b, Katoh *et al.*, 2005). In a few cases where amplifications could not be obtained, the respective fragment was coded as missing data. With distant outgroups, sequences from closely related taxa were used in the data matrix if the desired sequence was not available (e.g. *Eurytides asius* instead of *E. marcellus*). Overall, more than 56% of the sequences used were new. Some of the previously available *EF-1α* sequences were extended for 245 base pairs at the 5' end (Table 2.1).

Laboratory techniques

Total genomic DNA was extracted using the QIAGEN QIAamp DNA mini kit, and in all cases legs or thorax tissue were used. Polymerase chain reactions (PCRs) were conducted on either a T-gradient or a T-personal PCR thermocycler (Biometra GmbH, Germany), using a variety of primers, most of which have been described previously (Appendix 6). *Taq* Polymerase was added at the end of an initial 2-5 min denaturation at 95 °C, which was followed by 35 cycles of 94°C denaturing for 1 min, 45-52°C (depending on primer combinations) annealing for 1 min, 72°C extension for 1 min, and a final extension period of 72 °C for 7-10 min. PCR products were tested by electrophoresis on an agar gel, and if a single band was observed, were purified using a QIAGEN QIAquick PCR purification kit. If more than one band was present, the appropriately sized PCR product was cut from the gel and extracted using a QIAGEN QIAEX II gel extraction kit. Sequencing reactions were then conducted using Big Dye terminator cycle sequencing (Applied Biosystems, Foster City, CA) under manufacturer's recommendations. Sequencing products were filtered through Sephadex-packed columns and dried using a vacuum centrifuge. Final products were re-suspended in formamide and fractionated on an ABI prism® 377 automated sequencer.

All fragments were sequenced in both directions. Resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in Sequencher® 4.1 (GeneCodes Corp., Ann Arbor, MI). Consensus files were aligned using ClustalX 1.81 (Thompson *et al.*, 1997) and the alignment files were converted to nexus format with the aid of Se-Al 2.0 (Rambault, 2002). Initial multiple sequence alignments obtained from Clustal X with the default settings (gap opening = 10, gap extention = 0.20) were examined by eye and manually adjusted in some regions that contained gaps. *EF-1α*, *wg* and *ND1* had no alignment gaps, but several small indels were present in *COI+tRNA-leu+COII, ND5* and *16S*. The alignment of *16S* sequences was particularly problematic because of the presence of multiple indels. Other than known introns and previously reported indels (Zakharov *et al.*, 2004a; Katoh *et al.*, 2005), no other insertions or deletions were found in the alignments. In all analyses, all data were incorporated with no sites deleted. Individual datasets were assembled into a combined nexus file and analyzed in PAUP* 4.0b8-b10 (Swofford, 2002). Alignments, including the morphology partition, were subsequently deposited on TreeBase (www.treebase.org). MacClade 4.0 (Maddison and Maddison, 2000) was also used in addition to PAUP* to trace morphological character changes.

Phylogenetic analysis

Neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML), bootstrap, and decay analyses were all conducted in PAUP* 4.0b10 (Swofford, 2002). NJ was used for a preliminary rapid evaluation of sequences for each gene before assembling the combined data matrix, particularly to detect any possible sequence chimerism due to PCR contamination or sequence misalignment. Any questionable sequences were re-amplified and re-sequenced several times; this included *Baronia brevicornis*. Congruence in gene partitions on the final data matrix was tested using the partition homogeneity test of PAUP*, also known as incongruence length difference (ILD) test (Farris *et al.*, 1994). The test was conducted under parsimony with 100 random addition sequences of taxa and 100 replicates. Considering the unreliability of the ILD test (Graham *et al.*, 1998; Darlu and Lecointre, 2002), each data partition was also analyzed separately to determine alternative phylogenetic hypotheses. In all analyses, 6 taxa (*P. communis, H. phyleus, C. eurytheme, P. napi, P. acmon* and *C. tullia*) were forced as outgroups.

MP analysis. Parsimony analyses used heuristic searches, starting trees determined by 100 random taxon addition, tree bisection-reconnection (TBR) branch swapping algorithm, gaps treated as missing data, multistate characters treated as uncertainty, and all characters equally weighted. Parsimony hypotheses were evaluated by bootstrap analysis (Felsenstein, 1985) with 100 repetitions under the same parameters as for initial parsimony searches. Bootstrap searches were conducted for each data partition, combined mitochondrial and combined nuclear partitions, and the total data set. Decay values were calculated using the program TreeRot (Sorenson, 1999), and partitioned Bremer support (PBS) was also calculated for each combined set of partitions. I traced morphological character evolution for internal nodes on the combined phylogenetic reconstruction in PAUP* under the accelerated transformation (ACCTRAN) character-state optimization criterion.

ML analysis. A hierarchical likelihood ratio test was conducted using MODELTEST 3.0 (Posada and Crandell, 1998) to examine the fitness of 56 different evolution models to the partitioned and combined data, ranging from simple Jukes-Cantor to general time reversible (GTR) models. In each case, parameters from the best model were used in ML phylogenetic reconstruction, with the GTR plus gamma distribution plus invariable site (GTR+Γ+I) model found to be most appropriate in most cases. Independent ML analyses for each gene partition and combined data were then performed in PAUP* 4.0b10 for UNIX (Swofford, 2002). Support values for ML trees were estimated with 100 bootstrap replicates and, under the best fit model, with the ML tree selected as the starting seed.

Bayesian analyses. Bayesian posterior probabilities were calculated for partitioned and combined data sets using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001) under the GTR+Γ+I model and 4 (one cold and three heated) simultaneous Markov chains for 1,000,000 generations, starting with random initial trees and sampling every 100 generations. Substitution rates were estimated as part of the analysis from default priors, and model parameters were allowed to vary for analysis of the combined data. Trees corresponding to the burnin values estimated prior to initiation of each MCMC chain (varying from 800 to 5000, depending on gene partition) were discarded, and the majority rule consensus tree was generated using the remaining trees with posterior probabilities plotted on each node. The credibility of Bayesian support values for combined datasets has recently been questioned (Pickett and Randle, 2005; Mossel and Vigoda, 2005); thus the Bayesian posterior probabilities for partitioned and combined analyses were further contrasted with decay values obtained from the same trees.

Usefulness of genes. I investigated the relative utility of the genes I used for phylogenetic inference at various taxonomic levels from two perspectives. First, using PAUP*, I calculated the tree length (TL), log likelihood (-*ln* L), consistency index (CI) and retention index (RI) values for all of the phylogenies inferred in my analyses, with outgroups included. Second, I obtained uncorrected *p* distances with PAUP* for each gene partition and then plotted them against *COI*, with a saturation curve fitted to the data range. *COI* was selected for purposes of comparison, based on its extensive use in phylogenetic studies as well as in DNA barcoding (see www.barcodinglife.org). Average uncorrected *p* distances for *COI* between species, genera and tribes of subfamilies of Papilionidae used in this study were also calculated and plotted against a cumulative graph of all genes. Furthermore, for every gene I calculated the genetic distance between tribes in Papilioninae and Parnassiinae as well as their distance from Baroniinae.

DIVA analysis. The center of origin of the subfamily Parnassiinae has been previously suggested to lie "in the Turan arid zone which was located at E Tetis coast and included territories of the present day Aral Sea and Lower Syrdar'ya and Amudar'ya Rivers" (Korb, 1997: 1167). This hypothesis was tested through a dispersal and vicariance analysis with the aid the computer program DIVA (Ronquist, 1997). This method has frequently been used in reconstruction of ancestral distributions (given a phylogeny), including in swallowtail and nymphalid butterflies (e.g. Zakharov *et al.*, 2004b; Wahlberg *et al.*, 2005a). Outgroups and non-Parnassiinae species were excluded from DIVA analysis due to limited sampling; fossils were also removed due to uncertainty of their phylogenetic status, although their positions - as inferred in this study based on combined data - were plotted later on the DIVA reconstruction.

Areas selected in this study were based on the geographic distribution of the species but were also largely congruent with areas of endemism previously proposed for the western Palaearctic (Sanmartín, 2003). Areas included: *A*) North America, *B*) southern Europe, extending from Portugal and Spain to France, Italy, and Greece, *C*) northern Africa, *D*) the island of Crete [Kriti] in Greece, *E*) Anatolia, Lebanon and Israel, *F*) Zagros Mountains, extending from Iran to northern Iraq and SE Turkey, *G*) Caucasus mountains, *H*) the Iranian Plateau, including eastern Iran, Afghanistan, and the central Asian plains, *I*) the Himalayas as one unit, including Pamir, Tianshan, Tibet, Altai, etc., *J*) northern India, Bhutan, Bangladesh, Burma and northern Thailand, *K*) mainland China, Korea, and eastern Russia, and *L*) Japan.

Distribution data for each species were compiled in a nexus file in MacClade 4.0 as presence/absence for each region, with the ML phylogeny for all DNA used for the analysis. Analysis was conducted with and without restriction of maximum number of areas for ancestral nodes. DIVA assigns a cost of zero to vicariance (allopatric speciation) and duplication (sympatric speciation) events and a cost of 1 per unit area to any dispersal and extinction events; thus the best reconstructions are those that minimize the number of dispersals and extinctions under a parsimony criterion. Under the default setting, DIVA accumulates distribution areas towards the root, and the initial analysis produced an ancestral distribution of ABCDEFGHIJKL for the last common ancestor of Parnassiinae. Constraints of 2, 3, 4 and 5 unit areas were then imposed as the ancestral distribution, and the best construction (with the least number of dispersals = 26) was obtained when the maximum number of unit areas in ancestral distributions was set to three.

Molecular clock analysis. A likelihood ratio test (Felsenstein, 1988) was conducted in PAUP with the ML tree topology, and with and without enforcing a molecular clock to test the data for clocklike behavior among taxa. Divergence times were estimated using the r8S program (Sanderson, 2002) with semiparametric rate smoothing and a penalized likelihood approach applied to the phylogeny inferred from combined molecular data. The utility of the penalized likelihood approach in estimation of divergence times has been well demonstrated (e.g. Zakharov *et al.*, 2004a; Dumont *et al.*, 2005). Initial results were obtained under default settings with cross-validation enforced. The rate smoothing with the lowest cross validation scores was selected and the dating procedure was repeated. Standard deviations were obtained by bootstrapping data 1000 times using the seqboot module from PHYLIP 3.6 (Felsenstein, 1989) and with the aid of the r8s bootstrap kit (Eriksson, 2002). The constrained initial topology was used to re-estimate branch lengths for each node. This procedure was repeated for each tree, and the statistics were summarized using the "profile" command in r8S.

The molecular clock was calibrated using previously hypothesized divergence dates for taxa of Papilionidae as well as fossils and major geological events relevant to this study. Divergence dates proposed by Braby *et al.* (2005) for Troidini were tested separately due to age conflict with some of the other nodes. Dates used here are: 1) 82.5-89.1 MYA for the last common ancestor of Papilionini and Troidini (Gaunt and Miles, 2002), 2) 90 MYA for the initial split in Troidini (Braby *et al.*, 2005), 3) 64±6.8 for the split between *Troides* and *Parides* (Braby *et al.*, 2005), 4) 35-65 MYA for the initial split in the genus *Papilio* (Zakharov *et al.*, 2004a), 5)

39.8-45.1 MYA for the split between ancestral *P. machaon* and *P. demoleus* (Zakharov *et al.*, 2004a), 6) 9.6±1.2 MYA for the last common ancestor of *Luehdorfia* (Makita *et al.*, 2000). The relict distribution of *Allancastria cretica* was also used as a calibration point; separation of Crete from mainland Greece and Turkey has been estimated at 11 million years (Dennis *et al.*, 2000), although the island has existed in the form of several smaller islands until 3 million years ago due to tectonic uplifts of the southern Aegean (Papazachos and Kiratzi, 1996; Stöckhert, 1999). The speciation of *A. cretica* has also previously been hypothesized to have taken place before the Pleistocene (Olivier, 1993). I thus constrained the *A. cretica* node to 11-3 MYA.

The correct phylogenetic positions of fossil taxa used in this study are unclear; my morphological and combined analyses also fail to provide strong support for the position of these taxa due to the large amount of missing data, although the Miocene fossil *Doritites bosniaskii* has moderate support as basal to *Archon*. I therefore only used the upper limit of the Miocene (5.3 MYA) as the minimum age to constrain the split in ancestral *Archon*. Constraints were applied separately and in several possible combinations.

Results

Maximum parsimony analysis of 236 characters in the morphological dataset yielded 11 most parsimonious trees, the consensus of which is shown on Fig. 2.5. The resulting topology is mostly congruent with previous morphological hypotheses, in that *Luehdorfia* groups with other Zerynthiini, and *Hypermnestra* with

Parnassius. The positions of *Archon*, as well as two of the fossil taxa, remain uncertain as they fall into a polytomy. *Praepapilio,* however, sits basal to a monophyletic Papilionini, with weak support.

During initial analyses on molecular data, a previously published *ND1* sequence for *Parnassius clodius* (GenBank number U32464; Weller *et al.*, 1996) was found to belong to another species (*P. smintheus*). I deposited a new *ND1* sequence for *P. clodius* on GenBank (Table 2.1).

Base frequencies among in-group taxa were found to be significantly different (P=1.00) in all genes except *wg* (P=0.87), with mitochondrial genes having much higher A/T frequencies and *EF-1α* having slightly higher A/C frequencies. The ILD test demonstrated no heterogeneity among gene partitions (sum of lengths for original partition = 11438, *P* = 0.01). Exclusion of partitions one at a time also revealed no heterogeneity among remaining partitions (*P* = 0.01) in every case. Regardless, all analyses were performed both on partitioned and combined data to assess any undetected incongruence. Inferred phylogenies from the combined molecular data consistently demonstrated much better resolution and branch support values.

The total number of parsimony-informative characters in the combined dataset with outgroups excluded was 1877 (31.2%), with *wg* having the highest (40.3%) and *16S* the lowest (24.6%) proportion of informative sites in DNA sequence (Table 2.2). The morphology partition consisted mostly of parsimony informative characters (67.3%) within the in-group, due to my selective approach. In the combined mitochondrial (with outgroups, *tRNA-Leu* and *16S* excluded) and nuclear

DNA datasets, respectively 63% and 86.2% of informative characters were third codon positions.

The estimated model parameters were comparable to previous estimates for swallowtails (Zakharov *et al.*, 2004), with the GTR+Γ+I model found to be the best fit in every case except *ND1* (TIM +Γ+I). Both Modeltest and PAUP* predicted extremely high substitution rates for *COII* under GTR+Γ+I (best fit) and GTR+I models but not for other models. Examination of the character change ratio in the *COII* dataset under an NJ tree revealed relatively few G-T substitutions (only 1.15%). As demonstrated previously, a low G-T reference rate can lead to an overestimation of the other five relative rates under a GTR model (Zwickl and Holder, 2004). I further contrasted substitution rates obtained through Modeltest and PAUP* with those estimated freely in Mr Bayes under Bayesian criteria, and these did not seem to show any anomalies (Table 2.3).

Simplified tree topologies for partitioned and combined molecular data are shown in Fig. 2.6, and the support values for important nodes are listed in Table 2.4. Monophyly of Parnassiinae was supported mostly by Bayesian analyses, particularly by *ND5* and *wg* gene partitions. In maximum parsimony searches, Parnassiinae appeared as a monophyletic group only for *wg*. Heuristic searches conducted under the maximum likelihood criterion produced trees with monophyletic Parnassiinae for *ND5*, *EF-1α* and *wg*, as well as combined mitochondrial and combined nuclear data, although these were not supported (Bremer support = 0) and subsequently collapsed in bootstrap consensus trees.

Combined morphological and molecular data gave a tree with the same topology as the tree obtained from combined molecular data alone under the parsimony criterion, but with the addition of fossils and *Bhutanitis ludlowi*, for which no DNA sequences were available (Fig. 2.7). The sum of partitioned Bremer support for the MP tree from combined molecular and morphological data did not match the total Bremer support obtained from the same tree, and the supports estimated for fossil nodes were incorrect as TreeRot predicted support from molecular partitions for these taxa when there were no such data. The same problem was encountered in calculating Bremer support for Bayesian reconstruction of all data. It has been shown that shared missing data can serve as shared character states and consequently affect the support computation (Wilkinson, 1995; Makovicky, 2000).

Terminal branch lengths tended to be relatively larger in the ML tree based on combined mitochondrial data compared to those inferred from combined nuclear data, while the deeper node topology for Parnassiinae was identical in both trees (Fig. 2.8). In the combined nuclear dataset, partitioned Bremer support values from different genes were highly variable (Fig. 2.9). In many of my inferred phylogenies (Fig. 2.6, 2.7, and 2.8A), Papilioninae formed a well-supported group, as expected based on previous work (Caterino *et al.*, 2001, Zakharov *et al.*, 2004a). The position of *Baronia brevicornis*, however, was highly unstable, with all gene partitions, combinations and analyses reconstructing distinctly different phylogenetic positions for this taxon. Due to this labile behavior, most gene fragments for *Baronia* were re-sequenced to confirm the authenticity of the obtained sequences. In the combined

ML analysis of all molecular data, *Baronia* is sister only to Parnassiinae with relatively strong support (bootstrap 93, Bremer 20, Bayesian 1.0).

The results of my molecular and combined data provide only weak support for the monophyly of Parnassiinae (Table 2.4). However, Parnassiinae appear as a monophyletic group in most of my MP and ML heuristic searches as well as Bayesian reconstructions despite a lack of Bremer support, but collapse after bootstrapping. Nonetheless, three distinct, highly supported clades are observed within Parnassiinae: 1) *Hypermnestra* +*Parnassius*, 2) *Archon* + *Luehdorfia*, and 3) *Sericinus* + *Bhutanitis* + *Zerynthia* + *Allancastria* (Fig. 2.8, 2.9).

Usefulness of genes. Bar graphs of consistency and retention indices (Fig. 2.10) demonstrate notable variation among different genes, although there is little variation between different analytical approaches. Nuclear genes, particularly *wingless,* show the highest CI and RI and the lowest log likelihood values. Among mitochondrial genes, the lowest and highest CI values belong to *COI* and *ND1* respectively. *ND5* and *ND1* show lower RI values compared to other mitochondrial genes. Tree length and log likelihood, which are closely correlated, were highest in *COI*+*COII* and lowest in *16S.*

I tested patterns of relative divergence and saturation for each gene by plotting uncorrected *p* distances against *COI* (Fig. 2.11, 2.12). Three main categories of genes were evident: The first group (*COII*) demonstrates a divergence pattern virtually identical with that of *COI* for up to 15% divergence between taxa. The second group (*16S, EF-1α,* and *wg*) is initially clearly slower than *COI*, then gradually approaches the divergence of *COI* and intersects with it at about 9% (*wg*) to 12% (*16S* and *EF-1α*), and continues to diverge further after *COI* saturates (*16S* = 15%, *EF-1a* = 20%, and *wg* = 31% maximum divergence). The third group (*ND1*, *ND5*) initially shows similar rates of divergence to *COI* up to 7-9%, but continue to diverge to over 20% after *COI* saturates. Despite a much smaller sample size for Papilioninae, sequence divergences in my analysis were comparable to those previously calculated for *COI-COII* for that subfamily (Zakharov *et al.*, 2004a).

Comparison of uncorrected pairwise distances between tribes in Papilionidae and Parnassiinae, and among these subfamilies and the Baroniinae (Fig. 2.13) also demonstrated substantial variation among genes. Despite some overlap, all genes (except *16S*) consistently showed lower mean divergence between tribes in Parnassiinae compared to Papilioninae. The *Archon+Luehdorfia* clade was approximately equidistant from the other two clades in Parnassiinae, although with generally lower divergences than between the Parnassiini and Zerynthiini. In several cases, the medians of genetic distances between tribes in Papilioninae or Parnassiinae were higher than median of distances between subfamilies.

Divergence time estimation. The likelihood ratio test (Felsenstein, 1988) rejected a null hypothesis of rate constancy among taxa (δ=367, d.f.=41, P<0.0001). Semiparametric rate smoothing was therefore conducted using optimal smoothing with the lowest χ^2 value estimated through cross-validation analysis.

I found that three of my calibration points did not have any significant impact on age estimates (Table 2.5; Fig. 2.14). Even when they were not enforced, the resulting ages fell within the range proposed for the constraint; these included a minimum age for the ancestor of *Archon* (5.3 MY), the separation of Crete (3-11

MY), and the initial split in the genus *Papilio* (35-65 MY). Braby *et al.* (2005) critically review several previous divergence time estimates for Papilioninae and Troidini, and question the dates estimated by Gaunt and Miles (2002). Although their proposed age for the initial split in Troidini (*Battus* from other genera) at 90 MYA conflicts with the constraint for the last common ancestor of Papilionini and Troidini (82.5-89.1) (after Gaunt and Miles, 2002), I found that enforcing the estimated ages by Braby *et al.* in a separate analysis alongside other constraints did not have a substantive impact on ages estimated for Parnassiini (Table 2.5).

I also found that the estimated age of the node for the fossil *Thaites ruminiana* (~ 50 MYA) is somewhat older than the age of the fossil (Lower Oligocene, 30-38 MYA). Since the basal position inferred in this study for the fossil is also very weakly supported, *Thaites* may either form a distinct basal lineage for which apomorphies have not been preserved, or it actually belongs to the one of the clades *Luehdorfia+Archon* or *Bhutanitis/ Allancastria/Zerynthia*. Further studies on the fossil might provide new information and help clarify its phylogenetic position.

Discussion

The challenge of ranking. The ambiguity of the criteria by which taxonomic ranks above the species level are determined is a serious problem in systematics (Hennig, 1966). A standard system for biological classification, which would allow objective comparison of such taxa among various phyla, has yet to be developed (Avise and Johns, 1999; Williams *et al.*, 2001). One solution is to remove all indication of ranking from classifications, by naming and treating taxonomic names in accordance with a system such as the Phylocode (Cantino and de Queiroz, 2004; Holmes, 2004). However, the Linnaean hierarchy remains the accepted framework for most current taxonomic work, and so there is incentive to make this ranking system more consistent, at least within family-level taxa such as the Papilionidae.

A few fundamental elements have been considered in formally recognizing higher ranks: 1) monophyly, 2) geological age, 3) documentation of a decided gap, including genetic distance, separating the higher taxon from other taxa of the same rank, 4) taxonomic stability, and 5) inclusion of a consistent number of species (Hennig, 1966; Mayr and Ashlock, 1991; Mayr, 1999).

Monophyly is currently considered the single most important criterion to be satisfied in recognizing a taxon at a higher rank, whether this taxon is to be called a genus, a tribe, or a family (Hennig, 1966). If a higher taxon is found to be paraphyletic, it is generally split into smaller monophyletic categories that are diagnosable via synapomorphies (Mayr *et al.*, 1953; Peleggrino *et al.*, 2001). Such splitting may be welcomed – and encouraged – in the case of "monster genera" that include hundreds or thousands of species (e.g. Cobos, 1986; Thayer and Newton, 2005). Thus the number of species in a genus may also provide a basis for splitting that is not always fully articulated. In fact, most systematic arrangements are originally determined by the "experience, good judgment, and common sense" of a traditional taxonomist (Mayr and Ashlock, 1991).

It has been suggested that molecular distances can provide a "yardstick" for measuring the age of divergence between taxonomic counterparts at any level when traditional systematics falls short, although such rates can be very different between various classes of animals (Avise, 1994). Early molecular studies using protein and allozyme data generated much interest in this concept. More recently, percent DNA sequence divergence between species is often calculated but rarely used in refining generic or higher level boundaries (eg. King and Wilson, 1975); instead, such ranks are generally determined based on tree topology and branch support (e.g. Williams *et al.*, 2001).

Tradition also plays an important role in the designation of higher ranks. The International Code for Zoological Nomenclature (ICZN) has considered priority (and other principles) as subservient to stability in its preamble and in numerous articles (ICZN, 1999). Attempts to re-name or re-classify taxa that have been historically stable thus may be resisted by the scientific community unless there is convincing evidence that such a change is necessary.

I believe that my results demonstrate the need for a reclassification of Parnassiinae, even though this historically well-established group is generally considered to contain two tribes, Parnassiini and Zerynthiini. Instead, my results support recognition of three tribes: the Parnassiini, Luehdorfiini, and Zerynthiini (Fig. 2.9).

In my best supported phylogenies, four genera (*Sericinus, Bhutanitis, Zerynthia* and *Allancastria*) always form a well supported clade, with *Sericinus* as the basal species and *Bhutanitis* as the sister taxon to *Zerynthia+Allancastria*. This is congruent with a previously established taxonomic classification (Fig. 2.2) but without *Luehdorfia*. I use the oldest available tribe name **Zerynthiini** Grote, 1899 for this clade.

An important result of my study is the strong support for the clade *Hypermnestra+Parnassius*, an alliance that has been questioned in the past (e.g. Hiura, 1980; Häuser, 1993). In my molecular phylogenies, *Hypermnestra* nearly always appears either basal to *Parnassius* or is located within the *Parnassius* clade. This also reflects a widely recognized group (Fig. 2.2), but without *Archon*. I use the oldest available tribe name **Parnassiini** Duponchel, [1835] for this clade.

Although phylogenetic relationships within the genus *Parnassius* were beyond the scope of this study, my data support none of the previous hypotheses for the group (c.f. Hancock, 1983; Omoto *et al.*, 2004; Katoh *et al.*, 2005) except a few lower associations (*P. simonius* + *P. clodius, P. autocrator* + *P. delphius*). Further efforts should focus on more inclusive sampling of genes as well as more species of *Panassius*, since the currently available molecular data are unable to resolve the phylogeny of *Parnassius*.

The most unexpected outcome of my molecular analysis is the strongly supported association of *Archon* and *Luehdorfia* (Fig. 2.9). A molecular relationship between these two genera has been demonstrated previously, although with limited taxon sampling (Omoto *et al.*, 2004; Katoh *et al.*, 2005). In all of my phylogenies, these two genera either group together or collapse in a polytomy. This is particularly interesting because *Archon,* commonly known as "the false apollo", has long been considered a primitive *Parnassius* (e.g. Higgins and Riley, 1970). In addition, *Luehdorfia* has often been associated in one way or the other with *Bhutanitis* based on similarities in wing pattern elements (e.g. Ford, 1944a; Hancock, 1983), although recent studies on genitalia and early stages have challenged both of these assumptions (Saigusa, 1973; Igarashi, 1984; Stekolnikov and Kuznetsov 2003). In contrast to the molecular characters, I have found no morphological synapomorphies supporting a relationship between *Archon* and *Luehdorfia*; even the lack of larval tubercles indicated by Häuser (1993) is shared by most other Papilionidae.

I rely on molecular data to recognize the tribe **Luehdorfiini** Tutt, 1899 **stat. rev.** as established previously (Stekolnikov and Kuznetsov, 2003), but now consisting of *Luehdorfia* and *Archon*. My strongest support for this recognition is the topology of the combined molecular phylogeny. In order to quantify whether relative divergences provide support for erecting Luehdorfiini as a new tribe, I compared genetic divergences among tribes throughout the Papilionidae (Fig. 2.13). The uncorrected *p* distances from the *Archon*+*Luehdorfia* clade to both Zerynthiini and Parnassiini are similar, and yet somewhat lower than the distance between Zerynthiini and Parnassiini. These relative distances are incongruent with the consistent grouping of the *Archon*+*Luehdorfia* clade with the Zerynthiini in phylogenetic analyses. This pattern underscores the lack of homogeneity of divergence rates in the Parnassiinae. Consequently, genetic distances among the three clades of Parnassiinae are not very comparable to those between tribes in Papilioninae for most genes. Limited taxon sampling for the Papilioninae is another potential factor affecting the observed *p* distances. Thus the evidence for ranking the Luehdorfiini is ambiguous from the magnitude of molecular divergences within Parnassiinae. However, the monophyly of the *Archon*+*Luehdorfia* clade and the existence of gaps between tribal-level groups are distinctive features supporting recognition of Luehdorfiinae as a new tribe.

The strongly supported position of *Baronia brevicornis* as the sister to only the Parnassiinae in my ML phylogeny of all molecular data contradicts several previous studies (e.g. Hancock, 1983; Tyler *et al.* 1994) but supports a much older statement that *Baronia* "belongs in the neighborhood of *Parnassius*" (Jordan, 1907-1908). The genetic distance of Baroniinae from the other two subfamilies of Papilionidae (Fig. 2.13) does not show greater divergence than between Parnassiinae and Papilioninae, and hence does not support a basal position for *Baronia*. The estimated age for the separation of the last common ancestor of Baroniinae and Parnassiinae on my phylogeny is between 75-82 MYA, corresponding to the late Cretaceous when the North American and Eurasian landmasses were still connected (Dietz and Holden, 1970). It is possible that *Baronia* is the sole remaining member of an ancient sister lineage to Parnassiinae that survived the K/T mass extinction but did not diversify during the Tertiary (Labandeira *at al.*, 2002). Since the sparse sampling of Papilioninae in my study might have had an influence on the placement of *Baronia*, I refrain from further taxonomic conclusions on the position of the subfamily Baroniinae on the Papilionidae family tree.

Usefulness of genes. Several methods have been previously proposed for estimating levels of homoplasy and systematic information content provided by genes in a combined nucleotide data set. The most commonly employed methods include estimation of partitioned Bremer support (Baker and Desalle, 1997) and consistency and retention indices (Kluge and Farris, 1969; Farris, 1989). Other quantitative indices, such as rescaled consistency (Farris, 1989) and data decisiveness (Goloboff, 1991) also give insights into phylogenetic signal provided by each gene (Creer *et al.*, 2003; Danforth *et al.*, 2005).

Consistency and retention indices for my phylogenies provide evidence that the two nuclear genes consistently perform slightly better than mitochondrial genes (Fig. 2.10). Among mitochondrial genes, *COI* has the lowest agreement with its inferred phylogeny (lowest CI), but its ability to infer the proportion of potential synapomorphies retained on the phylogeny (RI) is better than *ND5* and *ND1*, both of which show higher CI values compared to *COI*.

The morphology partition in my dataset also shows very high CI and RI values from parsimony and Bayesian analyses, as might be expected for previously filtered data. It is interesting, however, that the CI for morphology is similar to that of nuclear genes. It should be noted that since outgroups were included in all trees used for estimation of CI and RI values, a saturation effect cannot be ruled out.

The partitioned Bremer support (PBS) values on my ML phylogeny of combined molecular data demonstrate substantial variation in the extent to which each gene partition contributes to the phylogeny (Fig. 2.9). Although PBS values indicate that none of the genes support or reject the monophyly of Parnassiinae, this node is moderately supported by *wg* as well as combined nuclear data in independent phylogenetic reconstructions and decay analysis (Table 2.4).

The utility of mitochondrial genes in phylogenetic analyses for lower taxonomic levels, and the strength of nuclear and ribosomal gene regions in providing phylogenetic resolution for deeper nodes, have been demonstrated previously (e.g. Simon *et al.*, 1994; Brower and Desalle, 1998; Caterino *et al.*, 2000). Comparison of uncorrected *p* distances in my data also shows that most protein coding mitochondrial genes (*COI*, *COII*, *ND1*, and *ND5*) show rapid increases in distances for recent divergences, but not for higher level relationships, although *ND1* and *ND5* continue to diverge at the generic level and above. On the other hand, *16S*, *EF-1α* and *wg* are more informative in generic- and tribe-level analyses, and *EF-1α* and especially *wg* can still provide resolution in phylogenies at the taxonomic rank of subfamily (Fig. 2.12).

Biogeography, genetic divergence, and character evolution. My dispersal / vicariance reconstruction and molecular clock analysis support a previous hypothesis (Korb, 1997) that the origin of Parnassiinae was in Central Asia. Optimizing the evolution of morphological characters at internal nodes using PAUP* under the MP criterion indicates that the common ancestor of Parnassiinae flew at lower elevations, was distributed from the Iranian Plateau to central Asia and China, and had larvae feeding on Aristolochiaceae (Fig. 2.14) (cf. Kreuzberg, 1994). However, a similar analysis using MacClade 4.0 suggests an equivocal state for the larval food plant of this ancestor, which could have been either Aristolochiaceae, Crassulaceae or Zygophyllaceae. My phylogeny also supports previous assumptions that this ancestral species would have had a pale yellow ground color, no tails, asymmetrical pretarsi (Ehrlich, 1958), a narrow and heavily sclerotized aedeagus, a heavily sclerotized ostial region in females, an elongate third segment of labial palpus, and an incurved middle discocellular vein on the forewing (Miller, 1987).

Based on maximum likelihood estimation of divergences (Fig. 2.14), the first split in ancestral Parnassiinae which gave rise to two lineages (Parnassiini and

Zerynthiini+Luehdorfiini) would have occurred after the initial collision of the Indian plate into Eurasia, which began around 65-55 MYA and continued until 54-42 MYA (Briggs, 2003). This event has been used to explain the diversification in Eurasian agamid lizards (Macey *et al.* 1999), coelacanths of the genus *Latimeria* (Springer, 1999; Inoue *et al.*, 2005), and *Tetraponera* ants (Ward, 2001).

Based on my character optimizations, the ancestor of Parnassiini, which perhaps resembled *Hypermnestra*, had solitary larvae; it also had cocooned pupae (Hancock, 1983), scaled antennae (Miller, 1987; after Hancock, 1983), sclerotized patagia, no mid-tibial spurs on hind tibiae, and a simple (=unforked) precostal vein on the hindwing (Hancock, 1983). The ancestral food plant of Parnassiini is also equivocal, although MP reconstruction suggests Crassulaceae as the ancestral larval host. The presence of many specialized characters in *Hypermnestra* is suggestive of a deep divergence with *Parnassius* and other Parnassiini, as supported by my molecular clock analysis (Fig. 2.14; Table 2.5).

Populations of the common ancestor of Parnassiini that remained in deserts could have adopted Zygophyllaceae as food plant and led to *Hypermnestra*, while those in higher altitudes began a rapid diversification under a new genus, *Parnassius*, with Crassulaceae feeding larvae. The ancestral *Parnassius* (node 18) in my analysis is estimated to be 34-39 MY old and to have originated in the Himalayas and China. My DIVA analysis shows that some species of *Parnassius* dispersed later into other parts of the world as far as Europe and North America, through a series of complex biogeographic and climatic events.

The uplift of the Tibetan plateau and formation of the Himalayas would have caused a complete geographic split in the range of the enigmatic *Aristolochia*-feeding ancestor of *Archon* and *Luehdorfia* about 42 MYA, leaving one lineage on each side of the high mountains. The fossil *Doritites bosniaskii,* which appears to be most closely related to *Archon*, demonstrates the expansion of an extinct ancestral linage into southern Europe. My DIVA analysis shows that the ancestor of *Archon* probably originated in the Zagros Mountains about 30 MYA and spread westwards into southern Europe and Israel. On the other hand, the lineage leading to *Luehdorfia* - which originated in China/Japan - switched to feeding on *Asarum.* According to Makita *et al.* (2000), the common ancestor of extant *Luehdorfia* appeared relatively recently at about 9.6±1.2 MYA, although I found that alternative molecular clock analyses without enforcing this date as a constraint show the split to have occurred as far back as 25-27 MYA (Table 2.5). Of the several phylogenetic hypotheses for the genus *Luehdorfia* (e.g. Takahashi, 1973; Saigusa, 1973; Hiura, 1978; Ishizuka, 1980, 1991; Shinkawa, 1991, 1999; Aoyama, 1994; Watanabe, 1996; Kato, 1998; Yashima, 1999; Matsumura *et al.* 2005), my results are most congruent with that of Makita *et al.* (2000).

It appears that larval gregariousness evolved in parallel with feeding on Aristolochiaceae in the Parnassiinae (Fig. 2.14). Troidini also show this trait as do Zerynthiini and Luehdorfiini. The *Aristolochia*-feeding ancestor of Zerynthiini that remained in China may have been similar to *Sericinus*; the larva had fleshy segmental tubercles with setae, the pupa was slender; the adult had no scales on tibia and tarsi (Miller, 1987; after Hancock, 1983).

A further speciation event about 31 MYA produced ancestral *Bhutanitis +Zerynthia+Allancastria*. DIVA analysis suggests that this species had good dispersal capability as it expanded its range into Iran and further into Europe. The lineage that remained in China would have produced *Bhutanitis* by 20 MYA. The results of my combined analyses also largely support a recent phylogenetic hypothesis for the evolution of the genus *Bhutanitis* based on *COI* alone (Zhu *et al.*, 2005).

By about 16 MYA, *Zerynthia* in Europe and *Allancastria* in the Zagros Mts diverged from a common ancestor. The ancestral *Allancastria,* which may have been similar to *A. louristana,* expanded its range through a series of dispersal events into southern Europe and the Middle East. The rich tectonic history of the Mediterranean (Steininger and Rögl, 1996) further assisted in speciation and produced today's complex distribution pattern of *Allancastria*, leaving *A. cretica* in Crete before its severance from the mainland about 8 MYA. A similar pattern of dispersal from Iran into Anatolia and Europe has been documented for Pachyderminae beetles (Sanmartín, 2003).

Conclusions

My results demonstrate that the current higher classification of Parnassiinae does not reflect the phylogeny of the group. I provide strong support for monophyly of three groups within the Parnassiinae, and weak support for the monophyly of the subfamily. Divergence times estimated using several previously established calibration points show that the initial diversification of genera of Parnassiinae occurred at about the same time that the Indian plate collided into Eurasia 65-42 MYA. These estimates correlate with other previously well-established dates of vicariance and tectonic events, although fossils mostly remain as unreliable sources for calibration due to uncertainties about their phylogenetic placement. Based primarily on molecular evidence, I propose the following sequenced classification of the subfamily:

Subfamily Parnassiinae Duponchel, [1835]

[? † Genus *Thaites* Scudder 1875]

Tribe Parnassiini Duponchel, [1835]
    Genus *Hypermnestra* Ménétriés, 1846
    Genus *Parnassius* Latreille, 1804

Tribe Luehdorfiini Tutt, 1896 **(stat. rev.)**
    Genus *Luehdorfia* Crüger, 1878
    † Genus *Doritites* Rebel 1898
    Genus *Archon* Hübner, 1822

Tribe Zerynthiini Grote, 1899
    Genus *Sericinus* Westwood, 1851
    Genus *Bhutanitis* Atkinson, 1873
    Genus *Zerynthia* Ochsenheimer, 1816
    Genus *Allancastria* Bryk, 1934

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Table 2.1. Taxa, collecting data, specimen identification, and Genbank accession numbers.

	Species	Locality (for specimens sequenced in this study)	Specimen ID	COI-COII	ND5	ND1	16S	EF-1α	Wingless
1	*Pyrgus communis* (Hesperiidae)	USA: CA: Solano County, NE Vallejo, 21.08.1997	FS-b-901	AF170857	DQ351044	U25880	DQ351078	AF173396	AY569043
2	*Hylephila phyleus* (Hesperiidae)	USA: CA: Berkeley, 27.08.1998	FS-b-989	AF170859	DQ351045	DQ351059	DQ351079	AF173398	DQ351124
3	*Coenonympha tullia* (Nymphalidae)	USA: CA: Oakland Hills	FS-b-984	AF170860	DQ351047	AF229952[1]	DQ351081	AF173399*	DQ351126
4	*Plebejus acmon* (Lycaenidae)	USA: CA: San Diego	FS-b-969	AF170864	DQ351046	DQ351060	DQ351080	AF173404	DQ351125
5	*Colias eurytheme* (Pieridae)	Canada: ON: Ottawa	FS-b-543	AF044024	DQ351048	U32456	AB194748[2]	AF173400*	AY569040
6	*Pieris napi* (Pieridae)	USA: CA: Redwood Canyon, Alameda Co., 28.06.1996	FS-b-943	AF170861	AB044594[3]	DQ351061	DQ351082	AF173401	AY569041
7	*Eurytides marcellus*	USA: FL: Ocala State Forest, 23.03.1988	FS-a-7	AF044022	AB088651[4]	-	DQ351087	AF044815*	DQ351128
8	*Graphium agamemnon*	SE Asia (Country Unknown); 12.1996	FS-b-900	AF170874	AB059508	DQ351062	DQ351086	AF173414	AY569046
9	*Iphiclides podalirius*	France: 1988	FS-a-6	AF170873	AB059546	AJ224087	DQ351088	AF173413*	DQ351129
10	*Battus philenor*	USA: VA: Bedford County	FS-a-3	AF170875	AB027573	AJ224086	DQ351083	AF173415*	DQ351130
11	*Parides photinus*	Costa Rica: Villa Colon, 03.02.[1988]	FS-a-149	AF170877	AB027578[5]	DQ351064	DQ351085	AF173417	DQ351127
12	*Troides helena*	Malaysia: 10.09.1997	FS-b-974	AF170878	AB084430	DQ351063	DQ351084	AF173418*	AY569047
13	*Papilio demoleus*	Malaysia: Penang Island, 16.05.1989	FS-a-68	AF044000	AB013159	AJ224099	DQ351090	AF044825	AY569114
14	*Papilio machaon*	France: Coudoux, 18.02.1988	FS-a-27	AF044006	AB013150	AB186206	AB186172	AF044828	AY569124
15	*Papilio thoas*	French Guiana: Pointe Macouria, 30.05.1990	FS-a-302	AY457601	DQ351049	DQ351065	DQ351089	AY457632	AY569126
16	*Baronia brevicornis*	Mexico:Teacalco, btw Guerrero-Morelos, 07.1988	FS-a-167	AF170865	DQ351050	-	DQ351091	AF173405*	AY569044
17	*Hypermnestra helios*	SE Kazakhstan: Ili River, Bakanas village, 1-15.05.1998	FS-b-1597	DQ351025	AB095659	AB186200	AB186166	DQ351106	DQ351131
18	*Parnassius phoebus* (I)	Canada: AB: Plateau Mt., 08.1986	FS-a-8	AF170872	AB063354	AB186173	AB186139	AF173412*	AY569045
19	*Parnassius hardwickii* (II)	E. Nepal	-	-	AB094969	AB186178	AB186144	-	-
20	*Parnassius schultei* (III)	China: Tibet, Trans-himalaya, Karola Pass, 22-28.06.1994	FS-b-1978	DQ351026	AB095619	AB186183	AB186149	-	-
21	*Parnassius tenedius* (IV)	Kirghizstan: Altai Mts., Aktash village, 16.05.1997	FS-b-1784	DQ351027	AB095658	DQ351066	DQ351092	DQ351107	DQ351132
22	*Parnassius delphius* (V)	Kirghizstan: Tian-Shan, Naryntoo Mts., 1-10.07.1996	FS-b-1775	DQ351028	AB095655	AB186185	AB186151	DQ351108	-
23	*Parnassius autocrator* (VI)	Tadjikstan: E. Pamir, Muzkoi Mts., W Morgav village, 08.2000	FS-b-1983	DQ351029	AB095634	AB186192	AB186158	DQ351109	DQ351133
24	*Parnassius simonius* (VII)	Kirghizstan: Transalai Mts., 1-20.07.1998	FS-b-1777	DQ351030	AB095649	DQ351067	DQ351093	DQ351110	-
25	*Parnassius clodius* (VIII)	USA: WA: Okanagen Co., Chinook Pass, 7.03.1986	FS-a-375	AF170871	AB095624	DQ351068	DQ351094	AF173411*	DQ351134
26	*Archon apollinaris apollinaris*	Iran: Kermanshah, Rijab, 9.04.1998	FS-b-2025	DQ351032	DQ351051	DQ351069	DQ351095	DQ351112	DQ351136
27	*Archon apollinaris bostanchii*	Iran: Lorestan, Ploedokhtar, 10.04.2003	FS-b-2063	DQ351033	DQ351052	DQ351070	DQ351096	DQ351113	DQ351137
28	*Archon apollinus*	Turkey: Oludinez, 9.04.1999	FS-b-1868	DQ351031	AB095661	AB186202	AB186168	DQ351111	DQ351135
29	*Luehdorfia chinensis*	China: Zhejiang, Lishui, 06.2002	-	AB179872	AB016826	AB071942	-	-	-
30	*Luehdorfia japonica*	Japan: Kanazawa, Ishikawa; emg. 20.02.1991	FS-a-335	AF170867	AB013142	AB186205	AB186171	AF173407*	DQ351138
31	*Luehdorfia puziloi*	Russia: Primorye, Vladivostok, 05.1999	EZ-2-11	DQ351035	AB013143	AB186204	AB186170	DQ351115	DQ351139
32	*Luehdorfia taibai*	China: Shaanxi, Qinling, 06.2002	FS-b-2102	DQ351034	AB016828	AB071944	-	DQ351114	-
33	*Sericinus montela*	Japan: Tanashi, near Tokyo, 4.04.1991	FS-a-399	AF170868	AB095665	DQ351071	DQ351100	AF173408*	DQ351143
34	*Bhutanitis lidderdali*	China: Yunnan, Dongchuan, 10.2002	FS-b-2044	DQ351038	DQ351053	DQ351072	DQ351099	DQ351118	DQ351142
35	*Bhutanitis mansfieldi*	China: Sichuan, East of Mei Mtn, 07.2000	FS-b-1589	DQ351036	AB026727	AB071945	DQ351097	DQ351116	DQ351140
36	*Bhutanitis thaidiana*	China: Sichuan, Daba Mtn, 07.2000	FS-b-1591	DQ351037	AB026728	AB071946	DQ351098	DQ351117	DQ351141
37	*Zerynthia polyxena*	Russia: District of Voronezh, 1-5.05.1998	FS-b-1596	DQ351039	DQ351054	DQ351073	DQ351101	DQ351119	DQ351145
38	*Zerynthia rumina*	Spain: Malaga; emg. 5.11.1989	FS-a-88	AF170870	AB095660	AB186201	AB186167	AF173410*	DQ351144
39	*Allancastria caucasica*	Turkey: Bolu Pro., Bolu Daglari, 21.04.2001	FS-b-2046	DQ351042	DQ351057	DQ351074	DQ351104	DQ351122	DQ351149
40	*Allancastria cerisyi*	Greece: Thessaloniki, 1990	FS-a-342	AF170869	AB095662	AB186203	AB186169	AF173409*	DQ351146
41	*Allancastria cretica*	Greece: Crete Island, Lassithi, 4.5.2003	FS-b-2038	DQ351041	DQ351056	DQ351076	DQ351103	DQ351121	DQ351148
42	*Allancastria louristana*	Iran: Lorestan, Malavi, 1000m, 4.04.1999	FS-b-2037	DQ351040	DQ351055	DQ351075	DQ351102	DQ351120	DQ351147
43	*Allancastria deyrollei*	Iran: West Azerbaijan, Takab, 2000m, 23.05.2003	FS-b-2086	DQ351043	DQ351058	DQ351077	DQ351105	DQ351123	DQ351150

1-5. Replacement sequences for fragments that could not be amplified: 1= *Coenonympha dorus*, 2= *Eurema hecabe*, 3= *Pieris rapae*, 4= *Eurytides asius*, 5= *Parides montezuma*.
* EF-1α sequences extended by 245 basepairs at 5' end.

Table 2.2. Summary of character partitions for protein coding genes (also by codon position), RNA genes, and morphology, with outgroups excluded.

Gene Partition	*COI*				*COII*				*COI+COII*				*ND5*				*ND1*			
Codon position	All	1st	2nd	3rd	All	1st	2nd	3rd	All	1st	2nd	3rd	All	1st	2nd	3rd	All	1st	2nd	3rd
Total characters	1547	516	516	515	684	228	228	228	2233	745	744	744	816	272	272	272	472	157	157	158
Constant	965	409	392	164	394	153	194	47	1361	563	586	212	434	170	213	53	226	80	122	24
Uninformative	105	24	33	48	72	13	17	42	177	37	50	90	106	29	26	51	70	25	15	30
Informative	477	83	91	303	218	62	17	139	695	145	108	442	274	73	33	168	176	52	20	104
% informative	30.8				31.1				31.1				33.6				37.3			

Gene Partition	*16S*	*tRNALeu*	*Ef-1 α*				*wg*				morphology	All data
Codon position			All	1st	2nd	3rd	All	1st	2nd	3rd		
Total characters	533	80	1240	413	413	414	404	135	134	135	236	6011
Constant	339	62	847	381	396	70	210	92	114	4	50	3511
Uninformative	44	8	78	13	8	57	31	15	10	6	27	623
Informative	150	10	315	19	9	287	163	28	10	125	159	1877
% informative	28.1	12.5	25.4				40.3				67.3	31.2

Table 2.3. Substitution model parameters[a] from partitioned and combined data sets estimated under ML and Bayesian approach and General Time Reversible model.

		Base Frequencies				Substitution rates							
Partition	Estimation	A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T	Γ	I
COI	ML	0.339	0.069	0.127	0.465	114.515	40.658	68.714	51.836	1732.026	1.000	0.468	0.473
	Bayesian	0.361	0.082	0.084	0.474	0.650	1.940	2.021	1.342	47.871	1.000	0.183	
COII	ML	0.401	0.060	0.079	0.460	6.965×10^{6}	2.648×10^{6}	7.628×10^{5}	2.412×10^{6}	3.543×10^{7}	1.000	0.396	0.381
	Bayesian	0.392	0.073	0.035	0.501	4.576	15.488	0.824	8.100	44.245	1.000	0.144	
COI+COII	ML	0.363	0.065	0.111	0.460	296.937	93.848	92.143	105.917	2859.617	1.000	0.441	0.451
	Bayesian	0.374	0.777	0.789	0.470	4.464	3.197	2.251	1.852	48.122	1.000	0.199	
ND5	ML	0.418	0.036	0.061	0.485	0.076	6.811	0.400	3.379	1.233	1.000	0.609	0.336
	Bayesian	0.415	0.014	0.056	0.515	1.181	15.899	0.308	34.691	13.839	1.000	0.215	
ND1	ML	0.395	0.078	0.110	0.417	0.149	7.770	1.939	1.878	1.362	1.000	0.492	0.171
	Bayesian	0.379	0.017	0.056	0.548	1.685	21.112	0.124	24.553	26.414	1.000	0.160	
16S	ML	0.462	0.081	0.055	0.402	0.052	11.354	1.988	0.581	0.521	1.000	0.523	0.500
	Bayesian	0.501	0.075	0.036	0.389	0.002	45.138	0.028	0.070	0.022	1.000	0.130	
All mtDNA	ML	0.411	0.046	0.062	0.482	3.110	10.176	1.686	7.864	30.261	1.000	0.506	0.445
	Bayesian	0.406	0.047	0.079	0.467	4.522	9.317	2.707	7.465	45.787	1.000	0.227	
EF1- α	ML	0.259	0.269	0.233	0.239	0.719	4.909	2.091	0.555	7.051	1.000	1.433	0.614
	Bayesian	0.252	0.268	0.233	0.246	0.921	6.302	2.483	0.711	7.906	1.000	0.187	
wg	ML	0.186	0.309	0.316	0.189	1.136	3.841	1.425	0.364	4.673	1.000	1.036	0.388
	Bayesian	0.165	0.317	0.333	0.185	1.979	9.241	3.267	0.500	7.837	1.000	0.163	
All ncDNA	ML	0.242	0.283	0.249	0.226	0.791	4.124	1.663	0.574	5.763	1.000	1.292	0.556
	Bayesian	0.238	0.291	0.245	0.228	0.812	4.601	1.748	0.628	5.581	1.000	0.218	
All DNA	ML	0.324	0.139	0.144	0.393	1.231	8.732	5.790	3.408	11.290	1.000	0.872	0.473
	Bayesian	0.315	0.147	0.150	0.390	1.242	8.882	6.223	3.141	10.771	1.000	0.268	

[a] Γ = α, estimated shape parameter; I = proportion of invariable sites.

Table 2.4. Support values for important nodes obtained from decay analysis (Bremer support, BR), bootstrap on maximum parsimony tree (BSMP), bootstrap on maximum likelihood tree (BSML), and Bayesian posterior probability (BPP). Values with asterisks are nodes that were present in heuristic searches, but collapsed in bootstrap consensus. S+B+A+Z represents *Sericinus, Bhutanitis, Allancastria* and *Zerynthia*. Bootstrap values are for 100 replicates, with the exception of BSML for *COI* (70 replicates).

	COI				COII				COI+tRNALeu+COII				ND5				ND1				16S				All mtDNA			
Node	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP
Papilionidae	5	55	75	1	0	<50	<50*	0.67	4	68	85	1	1	<50	<50*	0.94	3	<50	<50	0.52	4	53	56	0.92	20	99	100	0.99
Papilioninae	0	<50	<50	0	0	<50	<50	0	0	<50	<50	0	0	<50	<50*	0.85	0	<50	<50	0	0	<50	<50	0	8	70	90	0.99
Graphiini	5	<50	65	1	0	<50	<50	0	0	53	75	1	0	<50	<50*	0	2	<50	<50	0.84	0	<50	<50	0	7	86	100	0.99
Troidini	0	<50	<50	0	0	<50	54	0	2	<50	72	1	2	56	61	0.98	0	54	<50	0.69	1	<50	58	0.98	5	86	95	1
Papilionini	2	65	100	1	0	<50	56	0.58	4	68	100	1	0	<50	<50*	0	3	70	80	0.99	2	55	<50*	0.58	24	98	100	1
Troi+Papil	2	51	<50*	0.84	0	<50	<50	0	4	59	<50	0.99	0	<50	<50*	0	0	<50	<50	0.99	0	<50	74	1	11	97	100	1
Parnassiinae	0	<50	<50	0	0	<50	<50	0	0	<50	<50	0	0	<50	<50*	0.94	0	<50	<50	0.62	0	<50	<50	0	0	<50	54	0.65
Hyp+Parn	17	100	98	1	3	81	74	0.99	16	100	100	1	2	<50	<50*	0.83	5	74	84	0	4	91	66	0.96	17	100	100	1
Arch+Lueh	2	<50	64	0.95	0	51	73	1	4	71	91	1	5	70	88	1	3	66	<50	0	0	<50	<50*	0	15	100	100	1
S+B+A+Z	1	56	84	1	0	<50	<50*	0	5	74	89	1	3	<50	59	0.99	0	<50	<50	0.99	0	<50	59	0.93	13	93	100	1
Allan+Zeryn	11	100	100	1	3	79	<50	0	14	100	100	1	8	89	94	1	0	53	57	0.99	4	93	<50*	0	26	100	100	1

	Ef1a				wg				All ncDNA				All DNA				morphology				All Data			
Node	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP	BR	BSMP	BSML	BPP
Papilionidae	8	74	86	1	4	75	100	1	17	98	99	1	43	100	100	1	7	96	-	1	5	96	-	1
Papilioninae	0	<50	<50	0	0	<50	<50	0	0	<50	<50	0	0	<50	100	0	3	85	-	0.99	0	63	-	0.94
Graphiini	12	94	99	1	6	87	98	0.99	18	100	99	0	35	100	100	1	4	97	-	1	4	100	-	1
Troidini	3	52	71	1	0	<50	<50*	0.84	2	<50	81	1	16	95	100	1	6	67	-	1	5	96	-	1
Papilionini	8	91	84	1	12	100	99	1	16	100	100	1	38	100	100	1	2	73	-	0.86	1	95	-	0.86
Troi+Papil	0	<50	<50	0	0	<50	<50	0	0	<50	<50	0	16	95	100	1	0	<50	-	0	0	73	-	0.91
Parnassiinae	0	<50	<50	0	8	99	68	0.97	8	78	<50*	0.95	0	<50	86	1	0	<50	-	0	0	<50	-	0.93
Hyp+Parn	9	97	98	1	10	99	97	1	19	100	100	1	35	100	100	1	0	67	-	0	6	97	-	1
Arch+Lueh	10	100	93	1	6	95	84	1	8	100	98	1	47	100	100	1	0	<50	-	0	0	<50	-	0.70
S+B+A+Z	0	<50	92	0.92	4	84	86	1	8	81	94	1	27	100	100	1	0	<50	-	0	3	87	-	0.99
Allan+Zeryn	8	97	98	1	4	99	91	1	15	100	99	1	39	100	100	1	0	<50	-	0	13	99	-	1

Table 2.5. Age estimates (with standard deviations) in millions of years for internal nodes using calibration points shown in Fig. 14 and model-corrected branch lengths. Fixed nodes are shown in bold, constrained nodes are in bold italics. Constraints are: node 10 (82.5-89.1 MYA); *a* = node 13 (35-65 MYA); *b* = node 14 (39.8-45.1 MYA); *c* = node 29 (9.6±1.2 MYA); *d* = node 40 (3-11 MYA); and *e* = node 27 (min age=5.3 MYA). * = Fixed nodes after Braby *et al.*, 2005 (node11=90 MYA, node12=64.9±6.88 MYA), with all constraints imposed except for node 10 due to conflict of assumptions with that node.

	Node 10 fixed at 89.1 MY		Node 10 fixed at 82.5 MY		Node 11 & 12 fixed*,	Node 11 fixed*,	None fixed,
Node	No constraints	*a+b+c+d+e*	No constraints	*a+b+c+d+e*	5 constr. (n 10 free)	6 constr. (n 10 free)	6 constraints
root	111.37±0.57	111.37±0.57	103.13±0.53	103.14±0.52	113.17±3.1	112.48±0.57	103.23±1.52
2	107.27±2.40	107.27±2.46	99.30±2.21	99.33±2.26	108.73±2.61	108.38±2.45	100.02±1.95
3	75.97±18.75	76.27±18.61	69.92±12.16	70.45±17.38	73.88±18.28	77.23±18.72	69.00±16.52
4	51.58±19.46	51.86±19.32	47.66±17.66	47.76±17.89	47.79±18.82	52.12±19.39	42.31±17.07
5	48.82±19.90	48.72±19.96	44.73±18.08	45.08±18.38	47.41±18.05	49.25±20.15	46.09±16.89
6	104.04±2.49	103.98±2.53	96.23±2.28	96.30±2.32	104.832±2.68	105.04±2.58	96.62±2.41
7	73.93±17.45	73.49±17.31	68.72±16.33	68.20±16.11	72.67±16.90	74.47±17.50	67.09±16.72
8	47.49±18.54	47.48±18.80	44.25±17.54	43.45±17.11	46.35±18.30	47.98±18.92	45.54±18.36
9	100.87±2.39	100.90±2.43	93.23±2.13	93.40±2.24	102.22±2.65	101.90±2.51	94.07±2.34
10	**89.10±0.00**	**89.10±0.00**	**82.50±0.00**	**82.50±0.00**	98.50±2.58	98.59±2.71	***87.24±1.29***
11	61.53±15.72	61.58±15.87	57.32±14.88	57.09±14.06	**90.00±0.00**	**90.00±0.00**	60.03±16.02
12	39.06±16.54	39.11±16.17	36.41±15.32	36.09±15.14	**64.90±0.00**	***66.77±2.70***	38.96±16.57
13	57.93±14.86	***56.33±4.26***	53.63±13.66	***56.40±4.26***	***57.77±4.39***	***56.45±4.31***	***57.45±4.31***
14	36.21±15.21	***43.10±1.04***	33.19±13.87	***43.10±1.04***	***43.23±1.13***	***43.10±1.04***	***43.09±1.13***
15	81.22±11.71	82.34±10.78	75.26±10.85	76.56±9.98	83.47±10.37	83.46±10.76	77.73±8.94
16	64.32±12.21	67.12±11.29	59.34±11.37	62.52±10.30	68.26±10.95	67.69±11.53	63.33±11.06
17	50.16±13.22	52.07±13.04	46.48±12.06	48.51±11.98	52.45±11.16	52.70±13.14	49.30±11.83
18	37.59±12.17	39.03±12.15	34.83±10.96	36.22±11.29	39.14±10.69	39.81±12.02	36.90±11.27
19	27.97±10.04	28.89±10.11	25.99±9.21	26.84±9.36	29.68±10.28	29.58±10.04	27.14±9.13
20	16.89±8.55	17.53±8.74	15.55±7.86	16.06±8.11	18.53±8.03	18.02±9.06	17.60±8.79
21	20.52±8.85	20.83±8.93	19.22±8.17	19.59±8.29	20.88±8.95	21.50±8.91	20.13±8.68
22	14.37±6.91	14.39±6.77	13.48±6.49	13.15±6.26	14.42±7.06	14.92±6.80	13.44±6.06
23	9.20±5.60	9.15±5.37	8.68±5.32	8.64±4.96	9.09±5.25	9.53±5.53	8.01±4.40
24	9.27±5.42	9.51±5.63	8.77±5.10	8.87±5.21	9.60±5.83	9.75±5.57	8.70±5.03
25	50.79±12.23	54.64±11.28	46.83±11.07	50.87±10.38	55.88±10.77	55.20±11.51	52.71±10.67
26	37.86±12.45	42.72±11.41	34.87±11.36	40.06±10.44	43.53±11.37	43.12±11.57	41.85±10.98
27	27.18±11.40	***31.96±10.69***	24.68±10.43	***30.22±9.83***	***31.55±11.45***	***32.11±10.82***	***29.57±9.39***
28	16.89±8.55	19.96±8.95	15.52±8.01	18.80±8.24	20.51±10.68	20.18±9.12	18.40±8.95
29	27.54±10.90	***10.14±0.36***	25.42±10.10	***10.14±0.36***	***10.14±3.64***	***10.15±36.09***	***10.20±0.39***
30	18.96±8.61	7.07±1.79	17.47±7.99	7.06±1.83	6.94±1.66	7.07±1.77	7.10±1.76
31	12.07±6.71	4.56±1.78	11.14±6.24	4.50±1.76	4.46±1.81	4.60±1.78	4.63±1.84
32	40.26±11.24	43.85±10.67	37.12±10.38	40.93±9.86	44.69±9.65	44.29±10.79	41.81±9.57
33	31.47±9.86	34.71±9.71	28.96±8.96	32.56±8.97	35.61±9.02	35.15±9.75	32.59±9.13
34	20.27±7.94	22.42±8.08	18.78±7.04	21.03±7.44	24.72±8.97	22.88±8.13	22.69±8.66
35	12.47±6.30	13.65±6.72	11.66±5.82	12.90±6.23	15.43±7.90	13.78±68.02	15.53±8.03
36	24.16±8.63	27.26±8.62	22.07±7.60	25.59±7.94	28.08±8.11	27.62±8.63	24.93±8.11
37	15.95±8.11	18.12±85.69	14.58±6.74	16.97±7.97	18.51±7.38	18.09±8.54	16.18±8.37
38	17.78±7.68	20.96±7.68	16.19±6.92	19.73±7.09	21.58±6.66	21.23±7.70	19.16±6.67
39	12.57±6.54	15.64±6.57	11.58±5.95	14.85±6.11	16.43±5.49	15.89±6.59	14.55±5.89
40	8.88±5.30	***8.29±1.52***	8.22±4.91	***8.18±1.53***	***8.09±12.57***	***8.25±1.50***	***8.07±1.40***
41	5.92±3.82	5.49±1.80	5.46±3.56	5.44±1.81	5.03±1.69	5.46±1.82	5.07±1.78

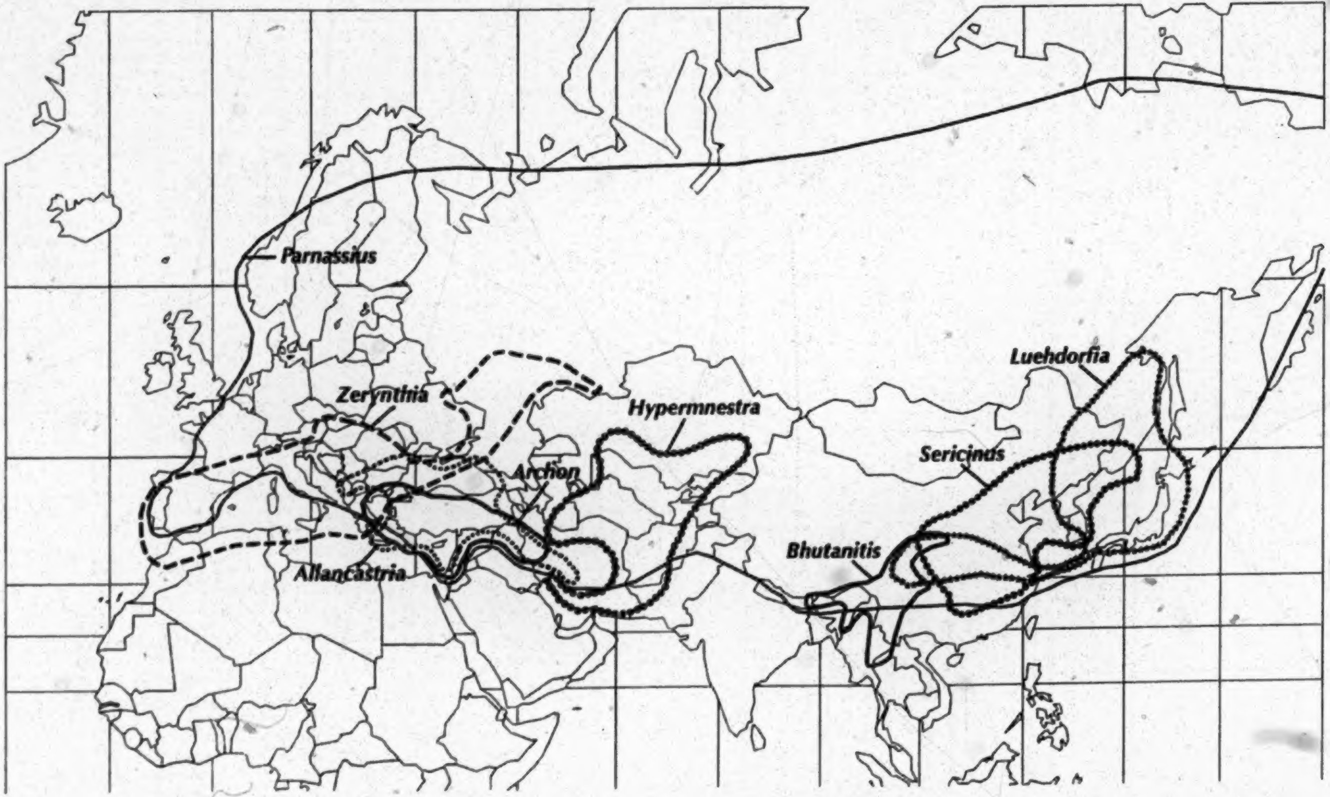


Fig 2.1. Geographical distribution of Parnassiinae genera in the Palaearctic (summarized from Kudrna, 2002; Nazari, 2003; Weiss 1991, 1992, 1999, 2005; Tschikolovets 1998, 2000, 2003; Hesselbarth *et al.*, 1995; Igarashi, 2003). The range of *Parnassius* also extends into western North America (Opler and Warren, 2003).

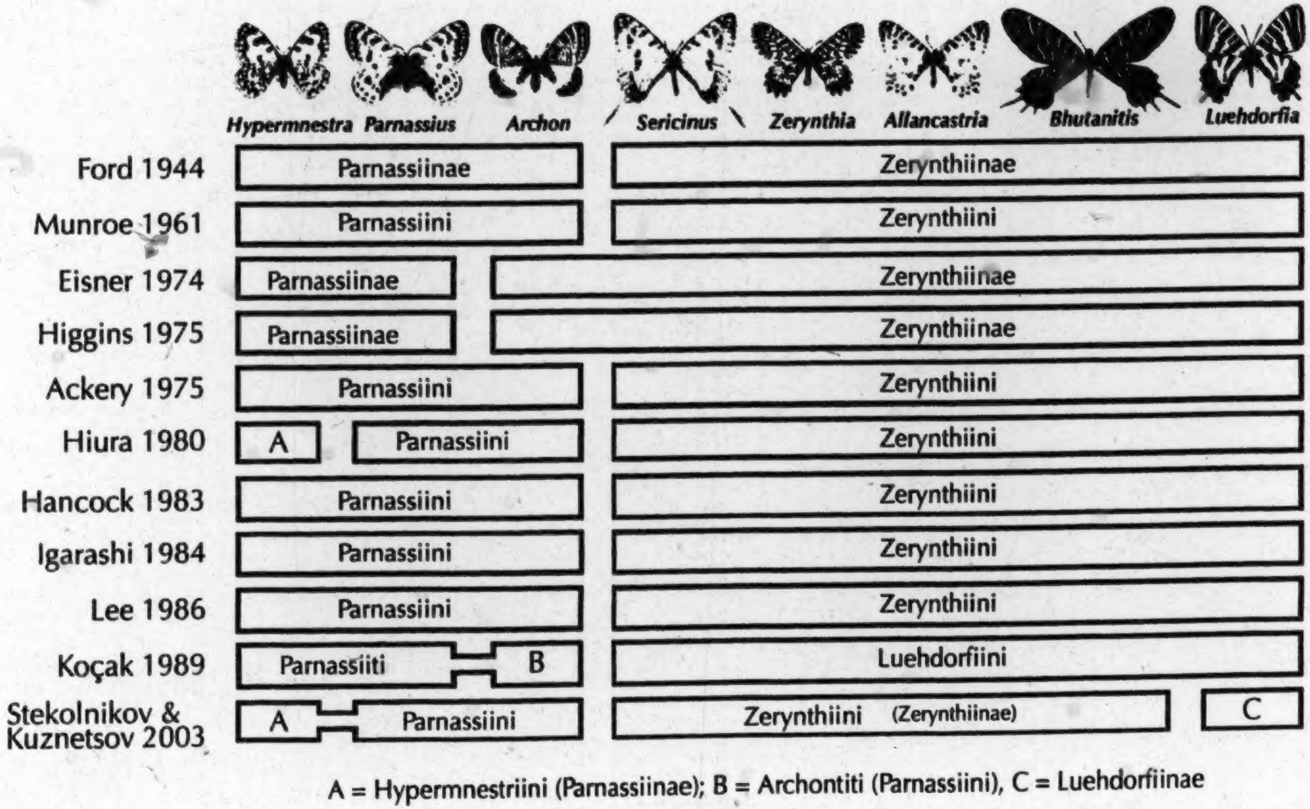


Fig 2.2. Previous classifications of Parnassiinae. Connected boxes indicate subdivisions within tribe Parnassiini (Koçak 1989) and subfamily Parnassiinae (Stekolnikov and Korshunov 2003).

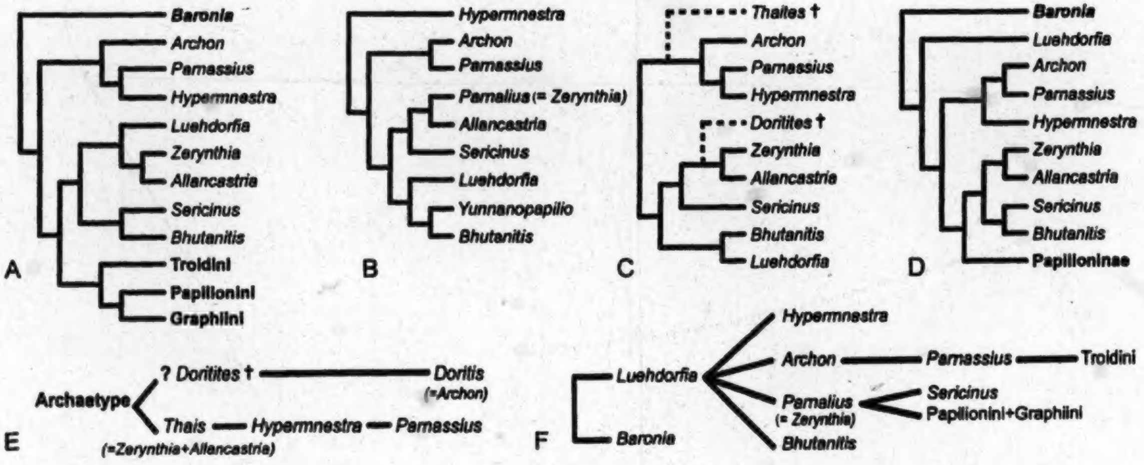


Fig 2.3. Previous phylogenetic hypotheses for Parnassiinae based on morphological characters; **A**. Ford 1944 (simplified), **B**. Hiura 1980, primarily based on wing pattern, **C**. Hancock 1983, **D**. Stekolnikov & Kuznetsov 2003, primarily based on genitalia, **E**. Le Cerf 1913, **F**. Igarashi 1984 (simplified), primarily based on immature stages.

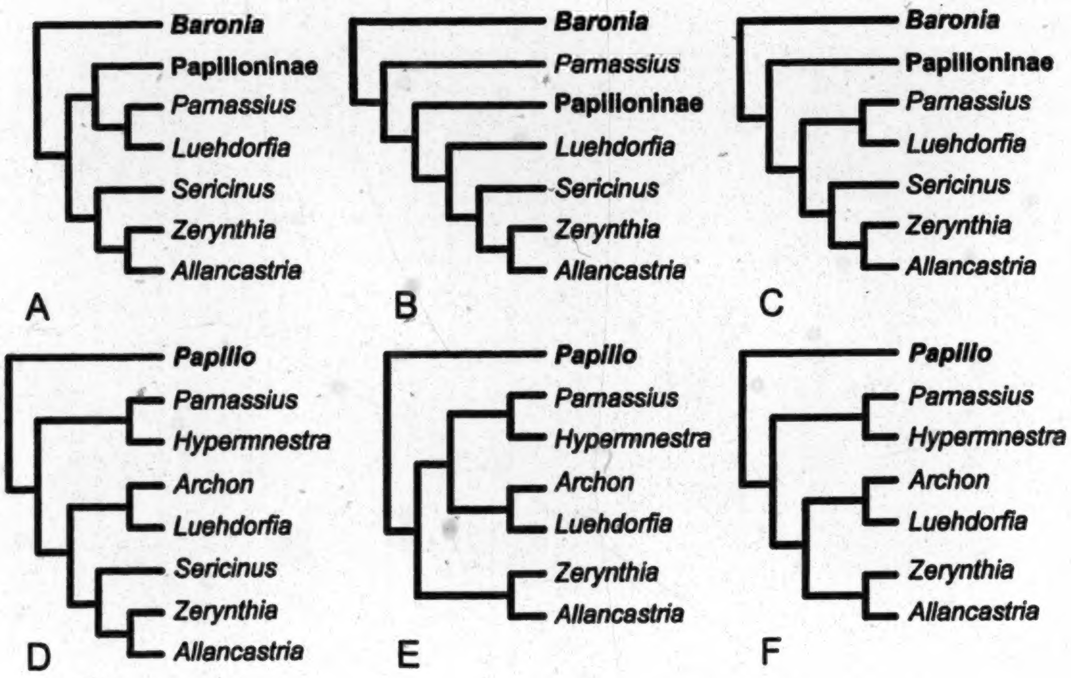


Fig 2.4. Previous phylogenetic hypotheses for Parnassiinae based on DNA evidence. **A-C**: Caterino *et al.* 2001, maximum parsimony trees based on *COI-COII* (A), *EF-1α* (B), and combined data (C); **D**: Omoto *et al.* 2004, neighbor joining tree based on ND5; **E-F**: Katoh *et al.* 2005, trees based on ND1 and 16S sequences, using minimum evolution *(E)*, maximum parsimony and maximum likelihood (*F*) methods.

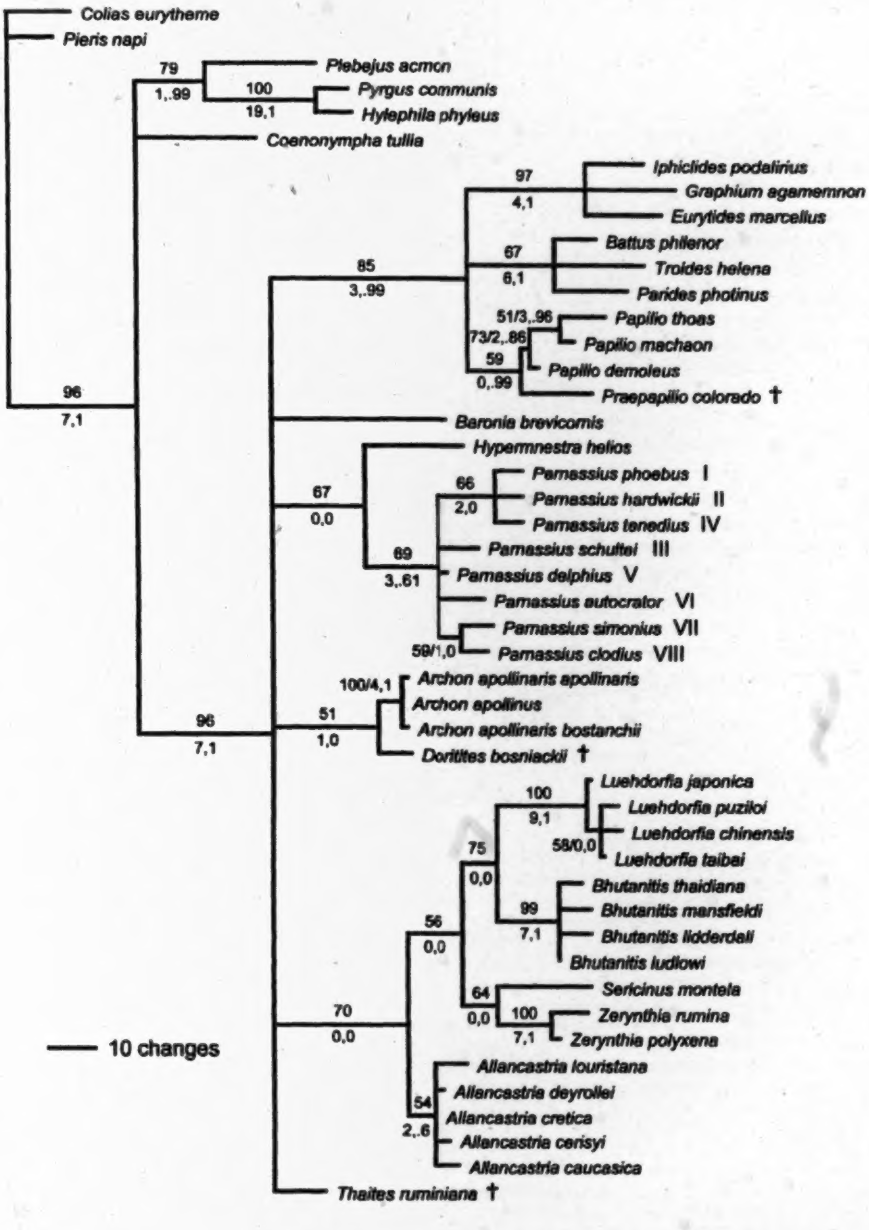


Fig 2.5. Maximum parsimony phylogeny based on 236 morphological characters, shown as the strict consensus of 41 most parsimonious trees (TL: 826, CI: 0.384, RI: 0.683). Bootstrap support values are plotted above and Bremer support/Bayesian posterior probabilities are below the branches. Neither MP nor Bayesian analyses on morphological data support monophyly for Parnassiinae.

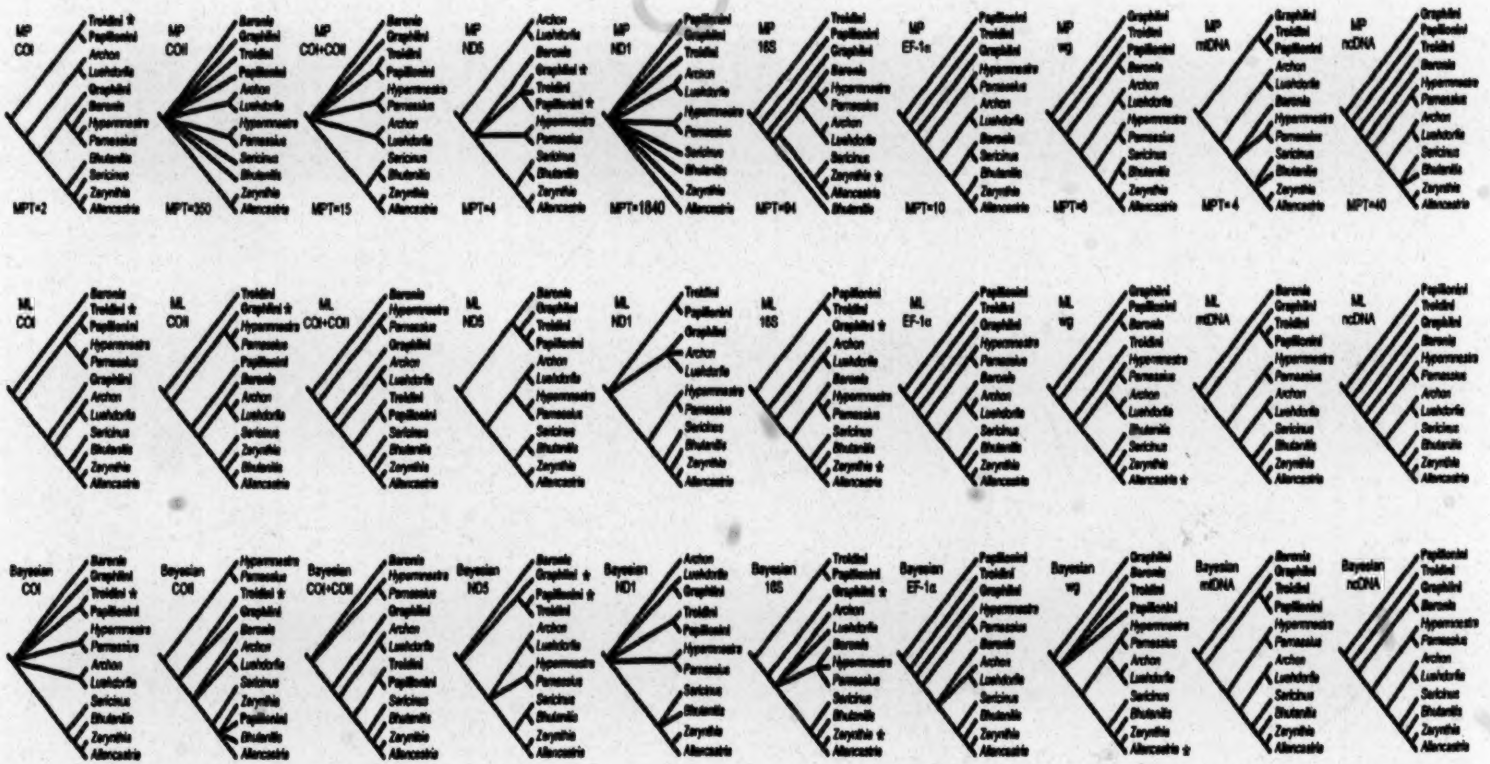


Fig 2.6. Simplified phylogenies resulting from MP, ML and Bayesian analyses on partitioned and combined data, with outgroups removed after analysis. For maximum parsimony analyses, consensus of the most parsimonious trees (MPT) is shown. Parnassiinae genera and Papilioninae tribes were monophyletic in all cases except where indicated by an asterisk (*); these taxa were paraphyletic with respect to their sister group.

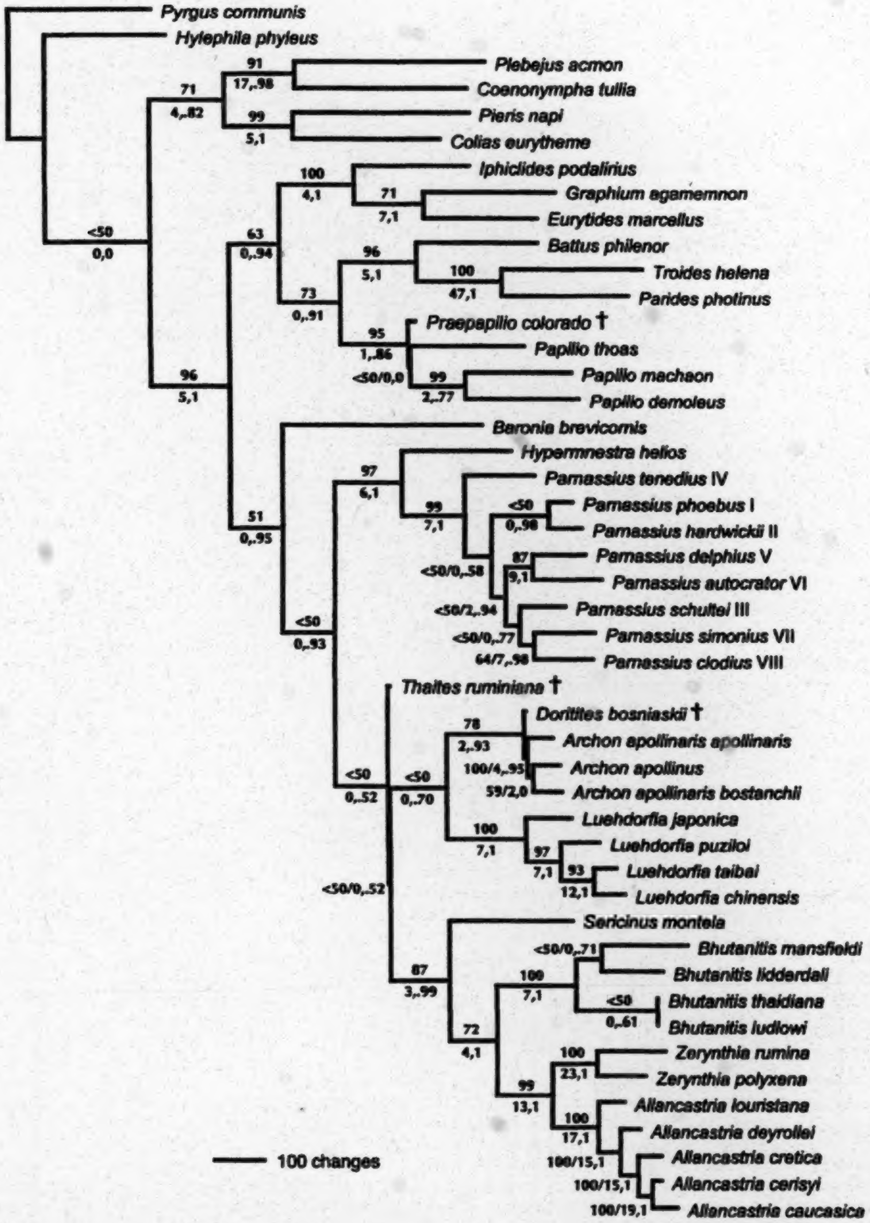


Fig 2.7. Tree from Bayesian analysis of combined molecular and morphological data (TL = 11712, CI = 0.353, RI = 0.516). Numbers above branches are bootstrap values from parsimony analysis; numbers below branches are total Bremer support and Bayesian posterior probabilities.

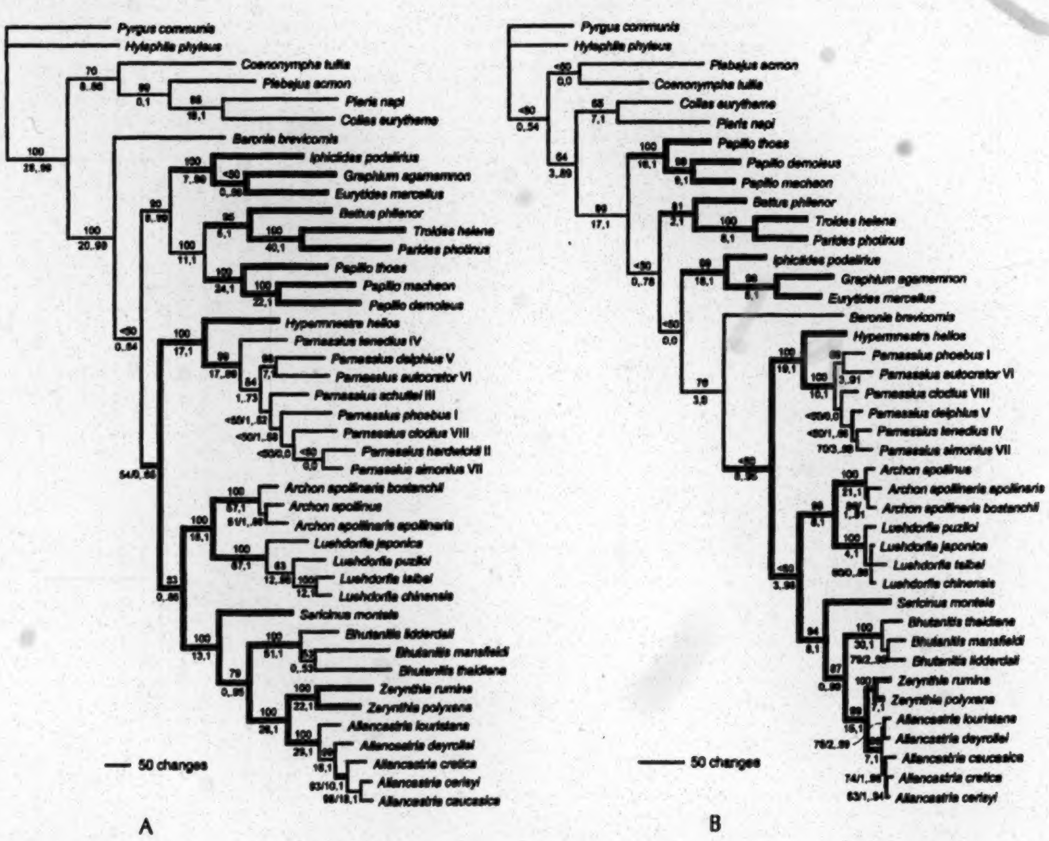
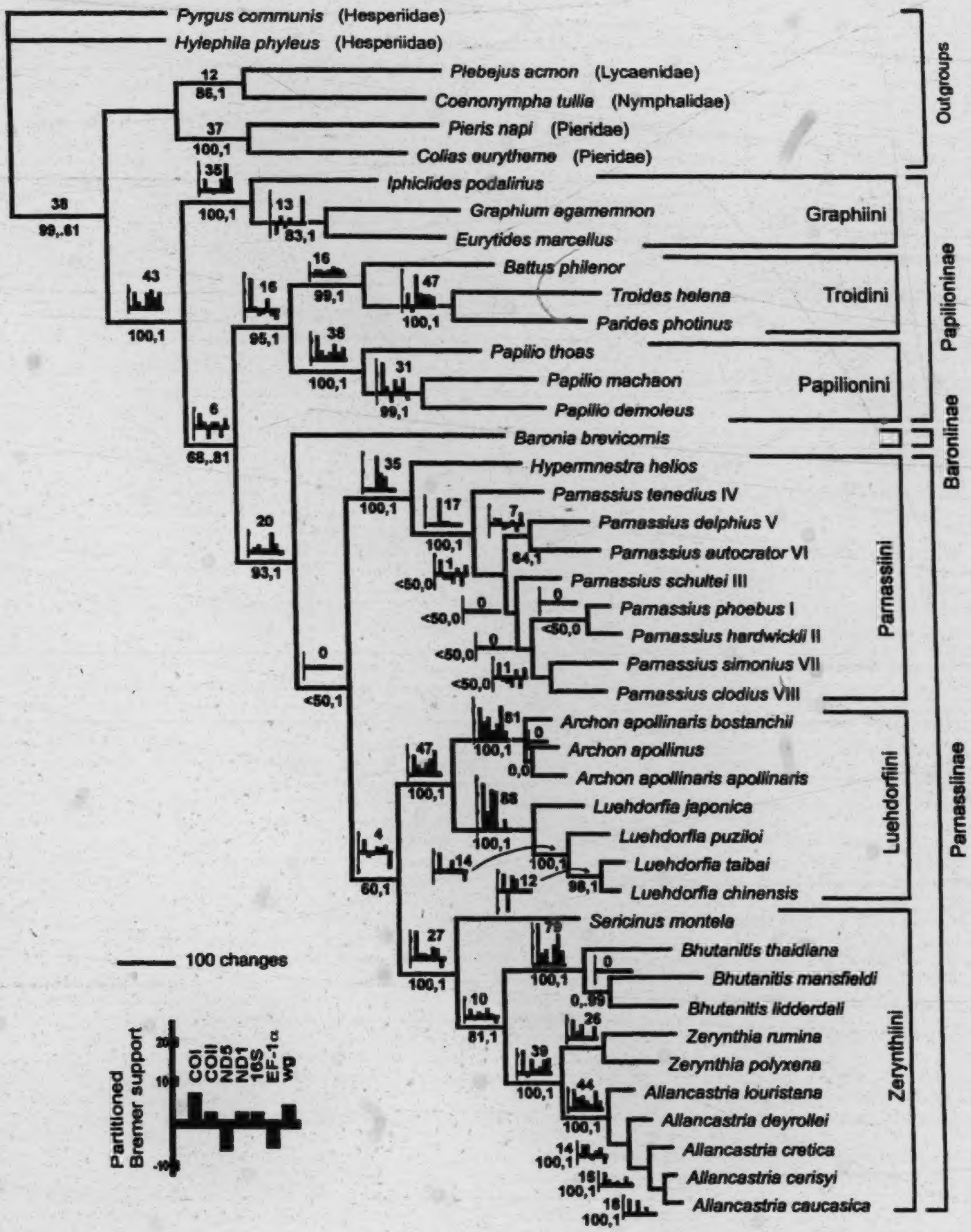


Fig 2.8. Maximum likelihood trees obtained for combined mitochondrial (*A*) (TL = 8043, CI = 0.327, RI = 0.461) and combined nuclear data (*B*) (TL = 2675, CI = 0.382, RI = 0.587), with bootstrap values plotted above and Bremer support values/Bayesian posterior probabilities below the nodes. In-group nodes shown with thick lines are shared in both phylogenies.


Pyrgus communis (Hesperiidae)
Hylephila phyleus (Hesperiidae)
Plebejus acmon (Lycaenidae)
Coenonympha tullia (Nymphalidae)
Pieris napi (Pieridae)
Colias eurytheme (Pieridae)
Iphiclides podalirius
Graphium agamemnon
Eurytides marcellus
Battus philenor
Troides helena
Parides photinus
Papilio thoas
Papilio machaon
Papilio demoleus
Baronia brevicornis
Hypermnestra helios
Parnassius tenedius IV
Parnassius delphius V
Parnassius autocrator VI
Parnassius schultei III
Parnassius phoebus I
Parnassius hardwickii II
Parnassius simonius VII
Parnassius clodius VIII
Archon apollinaris bostanchii
Archon apollinus
Archon apollinaris apollinaris
Luehdorfia japonica
Luehdorfia puziloi
Luehdorfia taibai
Luehdorfia chinensis
Sericinus montela
Bhutanitis thaidiana
Bhutanitis mansfieldi
Bhutanitis lidderdali
Zerynthia rumina
Zerynthia polyxena
Allancastria louristana
Allancastria deyrollei
Allancastria cretica
Allancastria cerisyi
Allancastria caucasica
Outgroups
Graphiini
Troidini
Papilionini
Papilioninae
Baroniinae
Parnassiini
Luehdorfiini
Zerynthiini
Parnassiinae
100 changes
Partitioned Bremer support
200
100
-100
COI
COII
ND5
ND1
16S
EF-1α
wg

(Fig. 2.9)

Fig 2.9. Maximum likelihood reconstruction of phylogeny of Papilionidae based on combined molecular data (TL = 10732, CI = 0.340, RI = 0.496). Graphs indicate partitioned Bremer support values for ingroup nodes, and the number above each graph represents the total Bremer support for each node. Numbers under each branch indicate MP bootstrap and Bayesian posterior probabilities from Bayesian analysis. The classification proposed in this study is shown to the right of tree.

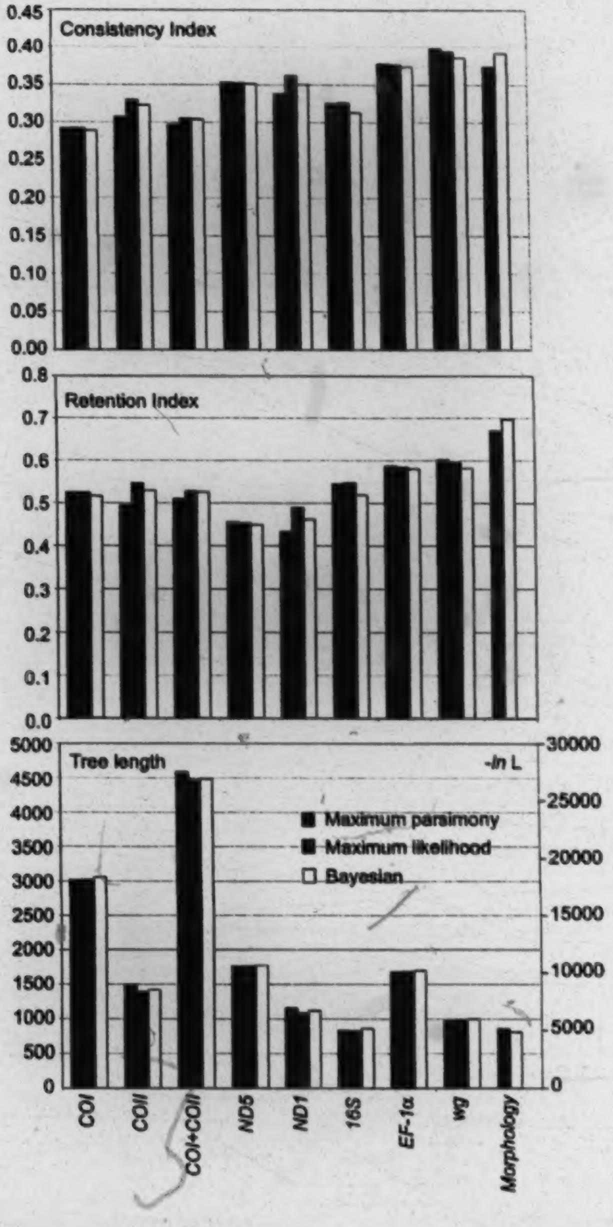


Fig 2.10. Distribution of consistency index (CI), retention index (RI), tree length and log likelihood (*-ln* L) values for genes and morphology based on trees derived from each data partition with all taxa and outgroups included.

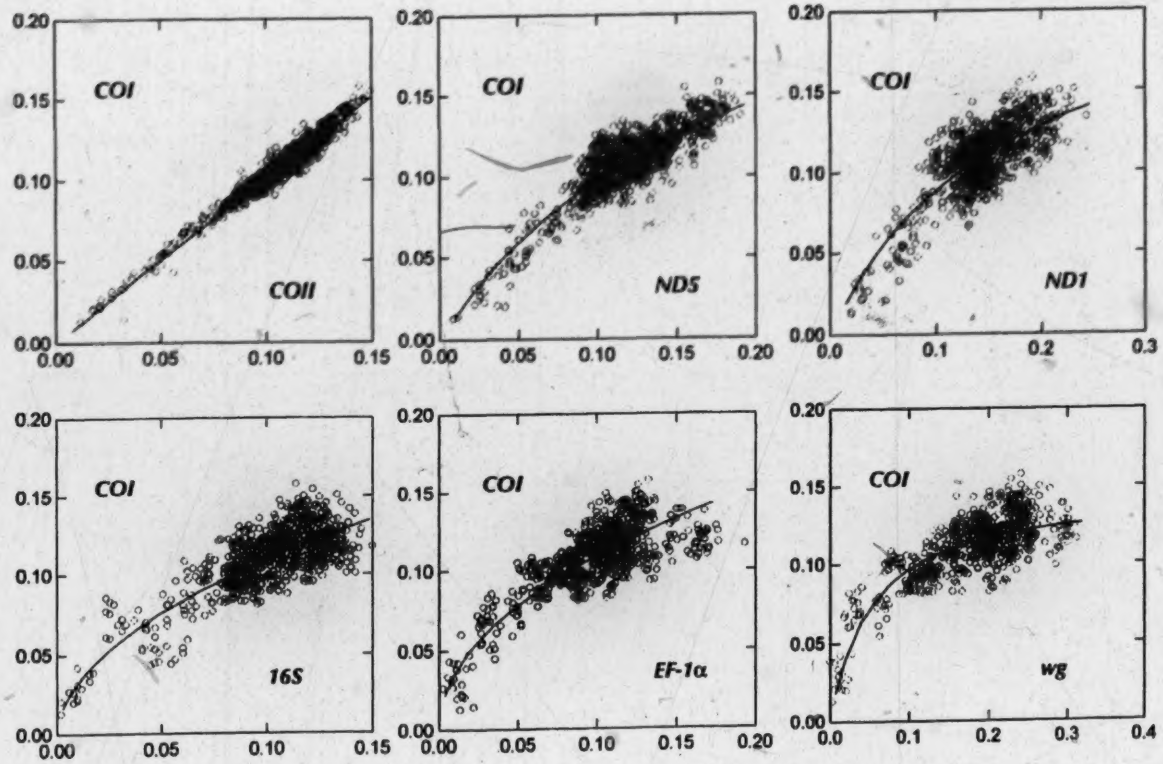


Fig 2.11. Scatter plots of uncorrected *p* distances for each gene (X axis) plotted against *COI* (Y axis). Saturation curves are fitted to the data and limited to the data range. Note the differences in scale of the X-axes.

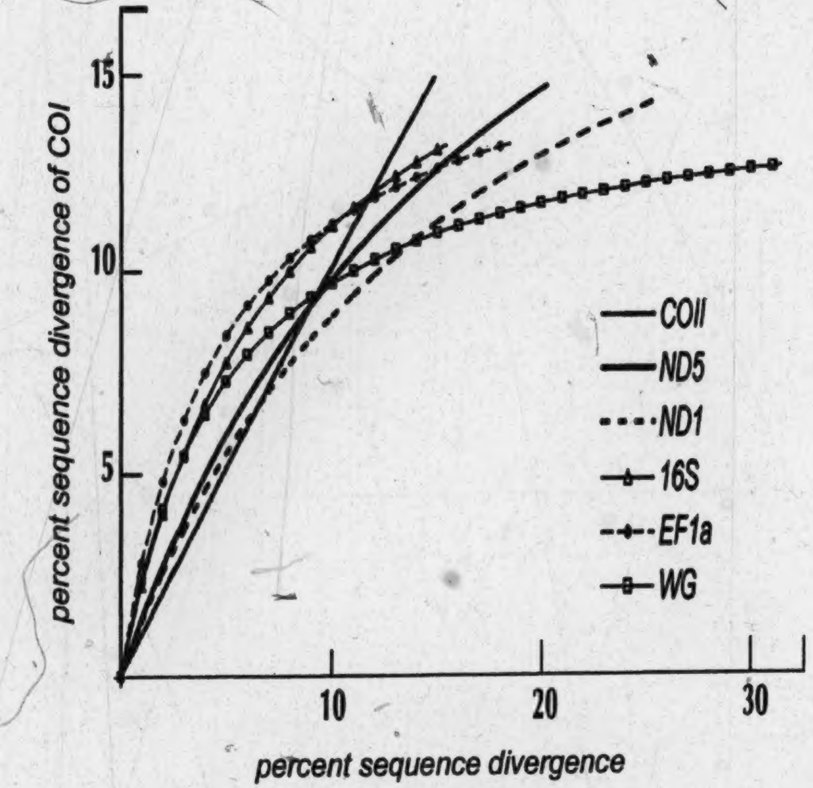


Fig 2.12. Overlaid saturation curves (from Fig. 2.11) of uncorrected percentage sequence divergence of genes in relation to *COI*.

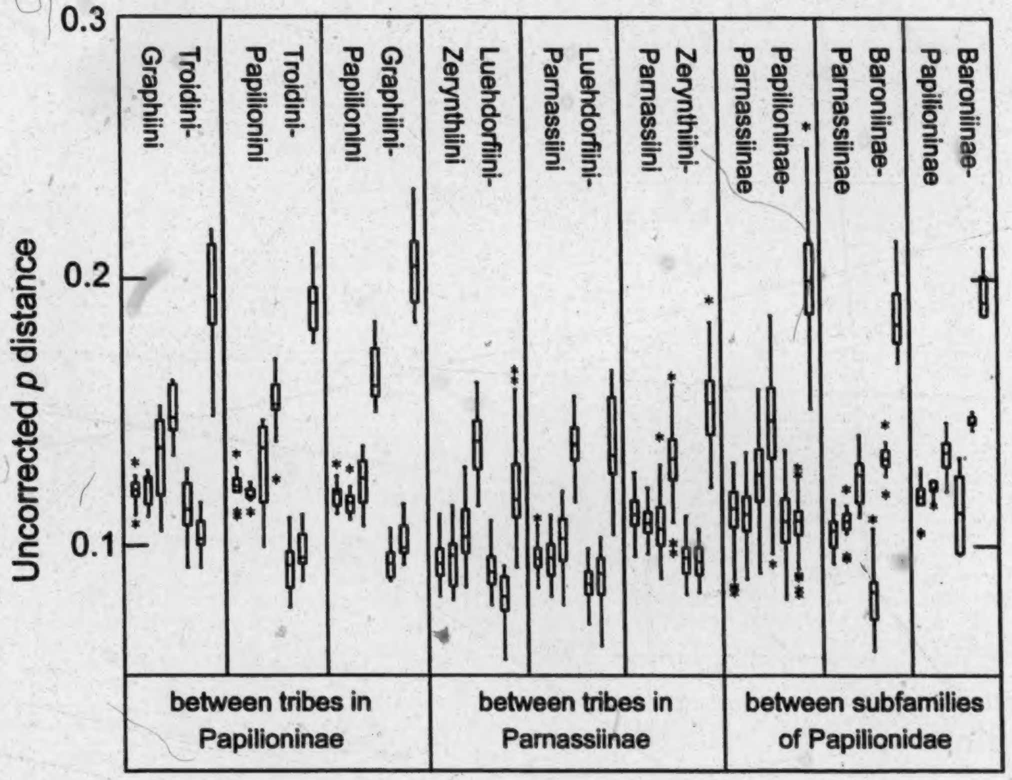


Fig 2.13. Uncorrected *p* distances between tribes in Papilioninae and Parnassiinae, and between subfamilies of Papilionidae. Bars in each section represent (from left to right) distances based on *COI, COII, ND5, ND1, 16S, EF-1α,* and *wg* sequence data, except for the last two panels where no *ND1* sequence for *Baronia* was available. The line in each box plot marks the median of the values; the length of the box shows the range within which the central 50% of the values fall; and whiskers show the range of values that fall within the inner fences (see SYSTAT 2005 manual for details). Outside values are shown by asterisks.

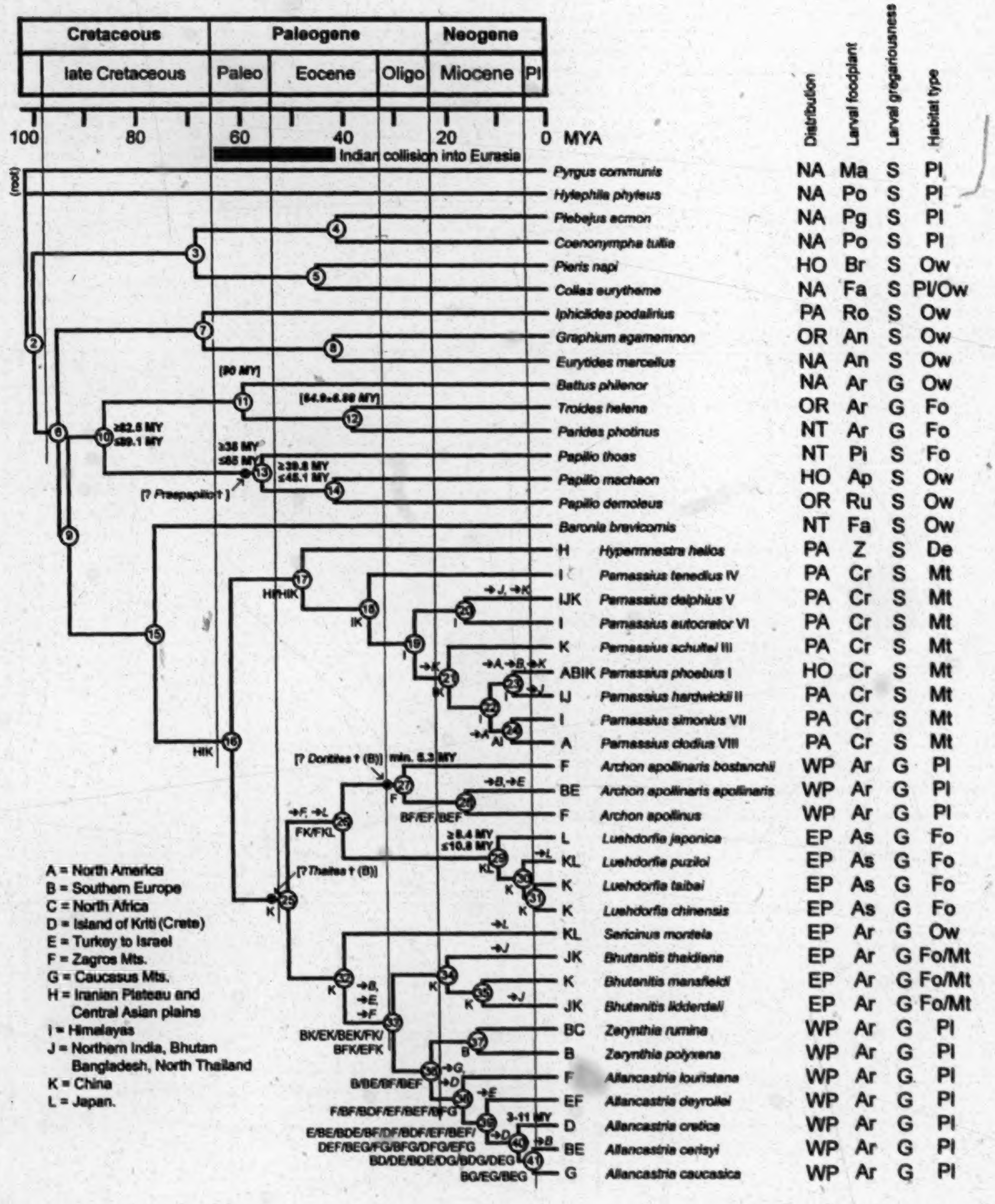
Cretaceous
Paleogene
Neogene
late Cretaceous
Paleo
Eocene
Oligo
Miocene
Pl
100
80
60
40
20
0 MYA
Indian collision into Eurasia
(root)
Distribution
Larval foodplant
Larval gregariousness
Habitat type
Pyrgus communis NA Ma S Pl
Hylephila phyleus NA Po S Pl
Plebejus acmon NA Pg S Pl
Coenonympha tullia NA Po S Pl
Pieris napi HO Br S Ow
Colias eurytheme NA Fa S Pl/Ow
Iphiclides podalirius PA Ro S Ow
Graphium agamemnon OR An S Ow
Eurytides marcellus NA An S Ow
Battus philenor NA Ar G Ow
Troides helena OR Ar G Fo
Parides photinus NT Ar G Fo
Papilio thoas NT Pi S Fo
Papilio machaon HO Ap S Ow
Papilio demoleus OR Ru S Ow
Baronia brevicornis NT Fa S Ow
H Hypermnestra helios PA Z S De
I Parnassius tenedius IV PA Cr S Mt
IJK Parnassius delphius V PA Cr S Mt
I Parnassius autocrator VI PA Cr S Mt
K Parnassius schultei III PA Cr S Mt
ABIK Parnassius phoebus I HO Cr S Mt
IJ Parnassius hardwickii II PA Cr S Mt
I Parnassius simonius VII PA Cr S Mt
A Parnassius clodius VIII PA Cr S Mt
F Archon apollinaris bostanchii WP Ar G Pl
BE Archon apollinaris apollinaris WP Ar G Pl
F Archon apollinus WP Ar G Pl
L Luehdorfia japonica EP As G Fo
KL Luehdorfia puziloi EP As G Fo
K Luehdorfia taibai EP As G Fo
K Luehdorfia chinensis EP As G Fo
KL Sericinus montela EP Ar G Ow
JK Bhutanitis thaidiana EP Ar G Fo/Mt
K Bhutanitis mansfieldi EP Ar G Fo/Mt
JK Bhutanitis lidderdali EP Ar G Fo/Mt
BC Zerynthia rumina WP Ar G Pl
B Zerynthia polyxena WP Ar G Pl
F Allancastria louristana WP Ar G Pl
EF Allancastria deyrollei WP Ar G Pl
D Allancastria cretica WP Ar G Pl
BE Allancastria cerisyi WP Ar G Pl
G Allancastria caucasica WP Ar G Pl
[90 MY]
[64.9±6.88 MY]
≥82.5 MY
≤89.1 MY
≥38 MY
≤55 MY
≥39.8 MY
≤45.1 MY
[? Praepapilio †]
HIK
H/HIK
IK
[? Doritites † (B)]
min. 5.3 MY
BF/EF/BEF
FK/FKL
≥8.4 MY
≤10.8 MY
[? Thaites † (B)]
BK/EK/BEK/FK/BFK/EFK
B/BE/BF/BEF
F/BF/BDF/EF/BEF/BFG
E/BE/BDE/BF/DF/BDF/EF/BEF/DEF/BEG/FG/BFG/DFG/EFG
BD/DE/BDE/DG/BDG/DEG
BG/EG/BEG
3-11 MY
A = North America
B = Southern Europe
C = North Africa
D = Island of Kriti (Crete)
E = Turkey to Israel
F = Zagros Mts.
G = Caucasus Mts.
H = Iranian Plateau and Central Asian plains
I = Himalayas
J = Northern India, Bhutan Bangladesh, North Thailand
K = China
L = Japan.

Fig 2.14. Maximum likelihood chronogram for Papilionidae based on all molecular data with no fixed dates and six constraints imposed (last column in Table 6). Calibration points are printed in bold; dates in brackets are from Braby *et al.* (2005) and have been analyzed separately. Inferred positions for fossil taxa are shown with solid dots. For Parnassiinae, dispersal events into new areas are shown with arrows. The most parsimonious reconstruction of dispersal/vicarience events, as shown above, required 26 dispersal events. Ecological characters are indicated to the right: Distribution (NA=Nearctic, HO=Holarctic, NT=Neotropical, OR=Oriental, PA=Palaearctic, WP=Western Palaearctic, EP=Eastern Palaearctic); larval food plant (Ma=Malvaceae, Po=Poaceae, Pg=Polygonaceae, Br=Brassicaceae, Fa=Fabaceae, Ro=Rosaceae, An=Annonaceae, Pi=Piperaceae, Ap=Apiaceae, Ru=Rutaceae, Z=Zygophyllaceae, Cr=Crassulaceae and Papaveraceae, Ar=*Aristolochia* [Aristolochiaceae], As=*Asarum* [Aristolochiaceae]); larval gregariousness (G=gregarious, S=solitary); and primary habitat type (Ow=Open woodland, De= Deserts, Pl=Plains, Fo=Forests, Mt=Mountains). Characters were coded after Ford (1944), Igarashi (1984) and Sillén-Tullberg (1988).

Chapter 3. Mitochondrial DNA variability and phylogeography in western Palaearctic Parnassiinae (Lepidoptera: Papilionidae): How many species are there?

Abstract:

I inferred the phylogeny and historical biogeography of Parnassiinae species from the western Palaearctic using an 825 bp fragment sequence of the mitochondrial protein-coding gene *cytochrome-oxidase I.* Investigation of genetic variation revealed several cases of overlap in extent of divergence between traditionally applied taxonomic ranks. In particular, I found deep divergences within populations of *Archon apollinus* from Turkey and Israel, *Zerynthia rumina* from Spain and North Africa, and *Hypermnestra helios* from Iran and Central Asia. Due to incomplete sampling and weak morphological support, I only report the possibility of the existence of more than one species within each of the above. The origin of ancestral *Archon* and *Allancastria/Zerynthia* is inferred to lie in the Iranian region, and diversification within genera is postulated to be the result of complex tectonic interactions between Eurasia and Africa during the past 20 million years which has involved multiple dispersal and vicariance events.

Introduction

The rich geological history of western Eurasia and its tectonic interactions with Africa and Arabia have had considerable effects on the evolution of many organisms inhabiting Europe and western Asia (Steininger and Rögl, 1996). Past tectonic events can often be traced in the phylogenies of life forms; the connection and disconnection of landmasses and seaways, emergence of new islands, and continuous formation of other physical barriers. These have resulted in repeated instances of vicariance and dispersal of species and created the composite biogeographic patterns seen today. Studies that correlate phylogenies with paleotectonic events may provide clues to geological events that have gone unnoticed (eg. Sanmartin, 2003; Guo *et al.*, 2005; Cosson *et al.* 2005). Among other organisms, butterflies have been subject to broad-scale research on biodiversity and contemporary geography in the region (e.g. Dennis *et al.*, 2000; Grill and Cleary, 2003; Schmitt *et al.* 2005).

The readily identifiable diversity that butterflies offer through their wing patterns has put them among the few groups that have been studied meticulously throughout modern history by a vast array of enthusiasts, from amateur collectors to professional biologists and taxonomists. Nonetheless, on a systematic level, lack of a clear definition for what constitutes a lower taxonomic category (species and subspecies) has had a considerable effect on butterfly taxonomy, resulting in checklists that are substantially different from one another. As an example, over the past century a multitude of "varieties" and "forms" of European Parnassiinae have been studied and named based on the slightest morphological differences; an extreme instance is *Zerynthia polyxena* (see Nardelli and Hirschfeld, 2002). These infra-specific names, which may be described based on limited specimens and only characters from their wing pattern, are subsequently sunk as junior synonyms of older available names (e.g. see Hesselbarth *et al.* 1995). On the other hand, some subspecies gain species-level recognition upon further examination of life history and internal morphology (e.g. *Archon apollinaris*; De Freina, 1985). The phylogenetic status of many such infra-specific names, however, remains unclear.

Geographically, swallowtail butterflies of the subfamily Parnassiinae are divided into an eastern Palaearctic (*Bhutanitis* [4 spp], *Luehdorfia* [4 spp], *Sericinus* [1 sp]) and a western Palaearctic group of genera (*Archon* [2 spp], *Allancastria* [5 spp], *Zerynthia* [2 spp], and *Hypermnestra* [1 sp]), although the genus *Parnassius* - which is not the focus of this study - is widespread in the Holarctic region with about 50 species. This east-west disjunction has been associated with the collision of the Indian plate into Eurasia about 65 million years ago (Nazari, 2006), which created the Himalayas and split the range of the last common ancestor of the subfamily. The number of infra-specific names described for the western group of species is larger than that for to the eastern Palaearctic group (for example, see Bryk, 1934, 1935).

The monophyletic genus ***Archon*** has two species: *A. apollinus* (Herbst, 1798) (type locality: Izmir, W. Turkey) distributed from Bulgaria to Greece and western Turkey, Syria, Palestine and Israel, and *A. apollinaris* (Staudinger, [1892]) (type locality: NE. Turkey) from eastern Turkey, west and northwest Iran, and northern Iraq. Both species depend on *Aristolochia* as larval host; they are morphologically similar, and the latter was separated as a distinct species through a comprehensive study of genitalia characters (De Freina, 1985), an idea that was further supported by comparison of early stages (Carbonell, 1991). At least nine other sub-specific names within *A. apollinus*, mostly described from Anatolia (e.g. Koçak, 1982) have been synonymized with the nominal subspecies by Hesselbarth *et al.* (1995), who list only three subspecies for Turkey.

Five species are recognized within the monophyletic genus ***Allancastria*** (Häuser *et al.*, 2005): *A. cerisyi* (Godart, 1824); *A. deyrollei* Oberthür, 1869; *A. cretica* (Rebel, 1904); *A. caucasica* (Lederer, 1864); and *A. louristana* (Le Cerf, 1908). All species feed on various *Aristolochia* at the larval stage. For a long time, the genus was known only with a single species, *A. cerisyi* (type locality: W. Turkey), with many local "forms" and subspecies being described under this name. Le Cerf (1913) was the first to re-evaluate whether these entities should be given specific status, prompted by his own discovery of *A. louristana* [in 1908] (type locality: W. Iran) and its striking similarity to *Hypermnestra*. He conducted a comparative analysis of many morphological characters of the adult as well as life stages of *A. louristana, A. apollinus, H. helios,* and *Parnassius tenedius*, trying to determine the phylogenetic relationship between them. A comprehensive review of *Allancastria* was later carried out by Bernardi (1970), who maintained the monotypy of the genus, listed every variety and locality known to that date, and described *A. cerisyi eisneri*. He also pointed out the co-habitation of two subspecies (*A. c. eisneri* and *A. c. speciosa*) in Jerusalem, hesitating to give them species rank. This situation was immediately noted by Larsen (1973) who assigned specific status to *A. deyrollei* (type locality: NE. Turkey) based on its co-existence with *A. cerisyi* in Lebanon. He also suggested that *A. louristana* should be a subspecies of *A. deyrollei*, and that *A. caucasica* (type locality: Georgia, Caucasus Mts.) should be given specific status as well. However, some of the publications in forthcoming years overlooked Larsen's suggestions.

Eisner (1974) and Ackery (1975) both maintained previous viewpoints that the genus was monotypic with several subspecies. Koçak (1975) described ssp. *abanti* for a population of *A. cerisyi* in northeastern Turkey, but Larsen (1976) suggested that this population is closer to *A. caucasica* than to *A. cerisyi*. Kuhna (1977) based on genitalia and wing morphology for the first time elevated *A. caucasica* to species level, suggested a specific status for *A. louristana*, and described two new subspecies for *A. cerisyi*. De Freina (1979) further reinforced the specific status and subspecies assignments of *A. caucasica*, *A. deyrollei* and *A. louristana*, and presented a detailed discussion on the biology of these species. The taxon *A. cretica* (type locality: Crete [Kriti], Greece) remained a subspecies of *A. cerisyi* until Koçak in 1981 gave it species rank. He also sank *Allancastria* as a subgenus of *Zerynthia*, which was accepted by some (e.g. Hesselbarth *et al.*, 1995) and ignored by others (e.g. Hancock, 1983; Miller, 1987). More recent studies by Carbonell (1996a, b), Hürter (2001) and others have provided a comparative analysis of many morphological and biological characters of the species of *Allancastria*, including life stages and male/female genitalia, as well as artificial hybrids, e.g. between *A. cretica* and *A. cerisyi* (Hürter, 2001).

The genus ***Zerynthia*** has two species: *Z. polyxena* (Denis & Schiffermüller, 1775) and *Z. rumina* (Linnaeus, 1758). *Z. polyxena* (type locality: Austria) has a wide distribution from southern, central and eastern Europe to southwestern Russia and Kazakhstan. There are 39 available subspecific names for *Z. polyxena*, with the highest diversity in Italy (Nardelli and Hirschfeld, 2002). The range of *Z. rumina* (type locality: S. Europe) extends from southern France to Spain, Portugal, Morocco, Algeria and Tunisia. The larvae of both species feed on *Aristolochia*. Previous work has demonstrated morphological differentiation between populations of *Z. rumina* within Africa and those in Europe (Tarrier *et al.*, 1994). There are at least 11 available subspecific names for the species, the majority of which refer to Spanish populations (Sabariego and Martinez, 1991), though two have their type localities in Africa (Binagot and Lartigue, 1998).

The genus ***Hypermnestra*** is monotypic, and its species, *Hypermnestra helios*, inhabits a narrow range of dry desert foothill habitats in Iran, Afghanistan, Pakistan, Turkmenistan, Tajikistan, Kazakhstan, Kirghizistan and Uzbekistan (type locality: Kazakhstan). The centre of origin of *Hypermnestra* has been suggested by Korb (1997: 1167) to lie "in the Turan arid zone which was located at E Tetis coast"; *H. helios* "strictly followed the distribution pathway [of its host plant] when spreading from the center of its origin". Korb (1997) also suggested that, subsequent to climatic changes in Central Asia in the Miocene, *H. helios* switched its food-plant as well as its flight pattern, while remaining on the plains. It is the only butterfly known to feed on *Zygophyllum* (Zygophyllaceae) at the larval stage.

I investigated the pattern of divergence within and between western Palaearctic species of Parnassiinae based on an 825 base-pair fragment of mitochondrial DNA, in order to find further evidence of their divergence and evaluate the rank of some of their currently used sub-specific names. In interpreting divergences, I particularly focused on geological events (within the past 10 MYA) that could have caused disjunctions and limited the opportunities for gene exchange between populations that are now on their way to complete divergence into separate species.

Materials and methods

Specimens. Specimens of Parnassiinae from the western Palaearctic were procured from as many localities as possible considering their availability (Table 3.1). Parnassiinae species from other regions were also represented in the analysis by one or two specimens to provide a basis for comparison. In the case of the genus *Parnassius*, only representatives from major species groups (after Omoto *et al.* 2004) were selected, with the exception of *Parnassius hardwickii* for which no specimens were available. Despite an exhaustive search, many critical populations of *H. helios, Z. polyxena,* and *A. apollinus* could not be sampled. DNA degradation also was a problem with some of the procured specimens.

The out-group, *Baronia brevicornis*, was selected as the closest sister taxon to Parnassiinae after Nazari (2006). All voucher specimens and extracted DNA samples are deposited in the E. H. Strickland Entomological Museum, University of Alberta. Photographs of voucher specimens and their collecting information can be viewed at http://www.biology.ualberta.ca/old_site/uasm//Vouchers/index.html. Remains of available specimens of *A. apollinus, Z. rumina* and *H. helios* were re-examined for morphological character variability. Male genitalia of all available specimens were prepared and examined. Wing pattern elements were also examined in all specimens, and previously published photographs (e.g. Bang-Haas, 1938; Wyatt, 1961; Hesselbarth *et al.*, 1995; Tshikolovets 1998-2003) were also checked in order to evaluate further variation.

Molecular techniques. Amplifications of 825 bp from from the 3'- end of the mitochondrial cytochrome oxidase subunit I (*COI*) were obtained for all taxa that had not been sequenced before, with the exception of one specimen (*A. cerisyi huberi* from Greece, FS-b-2078) for which only the first 402 nucleotides could be amplified. *COI* was selected based on its demonstrated phylogenetic utility in previous studies on swallowtail butterflies (e.g. Caterino & Sperling, 1999; Caterino *et al.* 2001, Vila & Bjorklund, 2004; Zakharov *et al.* 2004b; Matsumura *et al.*, 2005; Braby *et al.* 2005; Silva-Brandao *et al.*, 2005). All new sequences have been deposited on GenBank.

I extracted total genomic DNA using the QIAGEN QIAamp DNA mini kit, and in all cases I used tissue from legs or thorax of the specimens. Polymerase chain reactions (PCRs) were conducted on a T-personal PCR thermocycler (Biometra GmbH, Germany), using primers described previously (Table 3.2). For the most part I added *Taq* Polymerase at the end of the initial 2-5 min denaturation at 95 °C, which was then followed by 35 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 1 min, and a final extension period of 72 °C for 10 min. PCR products were then evaluated on an agar gel and purified only when a single strong band was observed, using a QIAGEN QIAquick PCR purification kit. Sequencing reactions were then conducted using an Applied Biosystems Big Dye terminator cycle sequencing kit (ABI, Foster City, CA). All fragments were sequenced in both directions. I filtered the sequencing products through Sephadex-packed columns and dried them using a speed-vacuum. Final products were re-suspended and fractionated on an ABI Prism® 377 automated sequencer. The resulting chromatograms were evaluated in Sequencher® 4.1; sequences were aligned in ClustalX 1.81 (Thompson *et al.* 1997), and converted to nexus format in Se-Al 2.0 (Rambault, 2002). Alignments were then evaluated by eye.

Phylogenetic analyses. Phylogenetic analyses were conducted for the most part in PAUP* 4.0b10 (Swofford, 2002) under neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) criteria. Heuristic searches were conducted for MP analysis under TBR swapping algorithm and with 100 random addition sequences. Bootstrapping of 100 replicates was conducted under the parsimony criterion with the default setting starting with a random seed and TBR branch swapping algorithm. The initial ML tree was generated using the parameters of the best-fit model (GTR+Γ+I) selected under Modeltest 3.0 (Posada and Crandell, 1998).

A second ML analysis was performed with topological constraints enforced to represent the tribal-level relationships proposed by Nazari (2006), using a tree file created in MacClade 4.0 (Maddison and Maddison, 2000). In order to test whether there was a significant difference between the two topologies, I used the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) in PAUP* with 1000 replicates and full optimization.

Bayesian analysis was conducted in MrBayes 3.04 (Huelsenbeck and Ronquist, 2001) under the GTR+Γ+I model and 4 (one cold and three heated) simultaneous Markov chains for 1,500,000 generations, starting with random initial trees and sampling every 100 generations. Substitution rates were freely estimated as part of the analysis from default priors. The burnin value was estimated prior to the initiation of the MCMC chain and the first 2000 trees were discarded. The majority rule consensus tree was generated using the remaining trees.

The pairwise distances were corrected in PAUP* with parameters from the best-fit model (GTR+Γ+I). Decay values for all trees were calculated using the program TreeRot (Sorenson, 1999). I also examined genetic divergences at various taxonomic levels by plotting the uncorrected *p* distances in a cumulative graph using SYSTAT 7.0.

Results

Despite numerous attempts, some specimens - including *H. helios* from Kopet Dagh mountains (Turkmenistan), *Z. rumina* from southern France, Portugal, and some Spanish populations, as well as *A. caucasica* from the Caucasus mountains - did not yield any usable DNA.

As expected for mitochondrial DNA (Simon *et al.*, 1994), the nucleotide base frequencies in my dataset were found to be significantly different (A=0.32, C=0.14, G=0.12, T=0.42; χ^2=47.5, P=1.000). Of the total of 825 base pairs, 538 were constant, 45 were parsimony uninformative, and 242 (29.33%) were parsimony informative, of which 49 were on first, 10 on second, and 183 on third codon positions.

The trees resulting from NJ, MP, ML and Bayesian analyses had somewhat different topologies, mainly in the deeper nodes. None of the trees reflected all the three tribes recognized for the subfamily proposed by Nazari (2006). ML analyses produced single trees, while MP analysis resulted in 840 equally parsimonious trees of 919 steps. Only Parnassiini (*Parnassius* + *Hypermnestra*) was consistently recovered in all analyses, and only the NJ analysis reflected Zerynthiini (*Sericinus* + *Bhutanitis* + *Zerynthia* + *Allancastria*) as a monophyletic group. Luehdorfiini (sensu Nazari, 2006) was never recovered as a clade, with *Luehdorfia* and *Archon* inconsistently appearing as sister to other Zerynthiini or Parnassiini genera. The position of *Sericinus* was also unstable through MP, ML and Bayesian analyses, often appearing as basal to other genera. Since the higher phylogeny was not of concern in this study, and the lower nodes were similar across these analysis, only the maximum likelihood tree is shown (Fig. 3.1). I also repeated the ML analysis with a constrained tree where three tribes (Parnassiini, Zerynthiini and Luehdorfiini) were fixed according to Nazari (2006) but all the shallower nodes were allowed to vary (Fig. 3.2). The Shimodaira-Hasegawa test showed that the constrained tree was not significantly longer ($P > 0.05$) than the unconstrained ML phylogeny ($\Delta -\ln L = 8.8544$, $P=0.183$).

Large divergences were noted between populations of *A. apollinus* from Israel and Turkey, *Hypermnestra helios* from Iran and Central Asia, and *Z. rumina* from North Africa and Spain, supported by high decay values. Average uncorrected pairwise (*p*) distances within and between species of Parnassiinae (Table 3.3), similarly showed a high degree of divergence (2.7%, 2.6% and 3.2% respectively) for the above cases, larger than the distance between many established species of Parnassiinae, e.g. *Archon apollinus* and *A. apollinaris*, or *A. cerisyi* and *A. caucasica*. To evaluate whether species-level recognition was justified, morphological characters were investigated in these three cases.

To examine species-level genetic variation within the subfamily, I plotted the uncorrected *p* distances within and between established species, between genera, and between tribes against the cumulative fraction of the values in a quadratic graph (Fig. 3.3). Overlap in values between categories was observed in every case. The few outliers in the "within species" distance category, corresponding to the species listed above (*Z. rumina*, *A. apollinus* and *H. helios*), mostly overlapped with the central 50% of the "between species" distance category.

Further examination of morphological traits in specimens from the unusually divergent populations of the above three species showed some differences in wing-pattern as well as in internal structures, but this variation was not comparable to the amount of divergence normally observed between different species in the subfamily. Most of the variable traits noted were those utilized by original authors in their descriptions of the subspecies in question. Most prominently, for *H. helios* I observed that the tip of the aedeagus had fewer teeth (1 or 2 per side) in Iranian populations compared to those from Central Asia (a series of 3 or 4 per side) (Fig. 3.4).

Discussion

Species definitions. The concept of what constitutes a species continues to be debated among systematists (Bock, 2004; Coyne and Orr, 2004; Hebert *et al.*, 2004; Queiroz, 2005). Some of the proposed species definitions incorporate an explicitly genetic component (Cracraft, 1989; Mallet, 1995; Sperling, 2003). A 2% sequence divergence in mitochondrial DNA is sometimes utilized as the benchmark for delimiting species, with the argument that intra-specific divergences are rarely greater than 2% (Avise, 1994; Hebert *et al.* 2003; but see Funk and Omland, 2003; Meyer and Paulay, 2005). However, it has been shown that some swallowtail species that are distinct by most conventional species definitions may show no divergence in mtDNA sequences (Hagen and Scriber, 1991; Sperling 1993, 2003). Such evidence against the utility of strict mtDNA cutoffs in species delimitations continues to accumulate for Lepidoptera. For example, genetic variation between some *Colias* species is less than 1% (DeChaine and Martin, 2005). On the other hand, a 3.8% differentiation in *COI* between the Australian subspecies of *Papilio demoleus* and others from Southeast Asia has been reported, without making any taxonomic decisions (Zakharov *et al.* 2004b). Other recent studies also provide evidence of substantial overlap in genetic divergence limits between higher taxonomic categories (Zakharov *et al.*, 2004a, Nazari, 2006). Although use of strict percent sequence divergence in drawing boundaries between species is generally disapproved of (Sperling, 2003; Meyer and Paulay, 2005; Rubinoff and Holland, 2005), unusually diverged mitochondrial sequences between populations of a species can nonetheless provide clues to speciation events that should further be investigated through characters from morphology and life history. Such divergences, however, should not be used as the sole criterion in delimiting species boundaries, or for that matter, any taxonomic category.

From a phylogenetic perspective, monophyly is now generally considered the single most important criterion for constituting any taxonomic category (Hennig, 1966; Mayr *et al.*, 1953; Mayr, 1999). If taxa are found to be paraphyletic, they are often split into smaller categories (Mayr, 1999). In my study, all traditionally recognized genera and species were monophyletic based on sequence from 825 bp of the *COI* gene, although my results do not recover the three tribes of Parnassiinae as proposed by Nazari (2006). The establishment of these tribes was based on a much larger dataset both in terms of genes and outgroup selection, and therefore it is not surprising that my inferred phylogeny based on a relatively short mitochondrial fragment does not reflect the higher classification of the subfamily. Furthermore, mitochondrial protein coding genes have faster divergence rates than nuclear genes that are generally used in phylogenetic analyses and also have more pronounced sequence saturation at higher taxonomic levels. This is supported by the fact that the *COI* uncorrected pairwise distances between the three tribes were observed to be very close to, and largely overlapping with, those between genera (Fig. 3.3).

Archon. At the species level, although extensive sampling across all subspecies of most species was not possible, there were several obvious cases of high divergences within species. First, I observed a large gap (2.7%) between the Israeli (ssp. *bellargus*) and the Turkish (ssp. *apollinus*) populations of *Archon apollinus*, which exceeded the average divergence between *A. apollinus* and *A. apollinaris* (1.9-2.3%). The taxon *bellargus* (type: Turkey, Hatay), together with many other names, has previously been synonymized with the nominal *apollinus* based on similarity of genitalia (De Freina, 1985). In light of present molecular evidence, I suggest that further work is needed to support this synonymy. Examination of specimens across other subspecies of the *Archon apollinus* "complex" should reveal further instances of high divergence within this species.

Branch lengths within the *Archon* clade (Fig. 3.1, 3.2) suggest that taxon *A. a. bostanchii* De Freina & Naderi (2003) from Iran, originally described as a subspecies of *Archon apollinaris*, is as diverged as *A. apollinus* or *A. apollinaris*, and might also prove to be a separate species when further evidence from its life history becomes available. My phylogenetic reconstructions suggest a basal position for this subspecies relative to the *A. apollinus* / *A. apollinaris* clade, but with weak support. This indicates a possible Iranian origin for *Archon*, with further dispersal to the west. Divergence of *Archon* from the most recent common ancestor of Luehdorfiini has previously been estimated to have taken place about 30 MYA (Nazari, 2006).

Allancastria. The topology of the *Allancastria* clade in my phylogenies is congruent with the one inferred by Nazari, (2006), with *A. louristana* as basal species, and *A. deyrollei, A. cretica, A. caucasica* and *A. cerisyi* branching off consecutively. I observed limited divergence within two of the species for which I had multiple samples (*A. deyrollei* = 0.8%, *A. cerisyi* = 0.5%). As for *A. deyrollei*, the Turkish populations (*A. d. deyrollei*) form a well-supported clade, and specimens from western Iran and Israel (*A. d. eisneri*) also group together but with weak support. Based on the ML phylogeny (Figs. 1, 2), the most parsimonious hypothesis for dispersal of *A. deyrollei* is that the ancestral stock dispersed from Iran to Turkey and Israel.

The short branch length and consistently close alliance of *A. caucasica* with *A. cerisyi* in all of my phylogenies do not support separate specific status for *A. caucasica*, and mtDNA of this species seems to be part of the larger variation within

A. cerisyi. Previous work on morphology and genitalia (Kuhna, 1977), as well as the biology of *A. caucasica* (Carbonell, 1996) have demonstrated differentiation of *A. caucasica* from *A. cerisyi.* A recent molecular study (Nazari, 2006) using multiple nuclear and mitochondrial genes (including *COI*) also found strong support for alliance between the two species. *A. caucasica* flies together with *A. cerisyi* in many localities in Turkey (Hesselbarth *et al.*, 1995). Although the limited divergence observed between *A. caucasica* and *A. cerisyi* could be a sampling artifact of the short length of the DNA fragment examined in this study, I suggest further work is needed to evaluate the separate specific status for *A. caucasica.* The ancestral distribution of the *A. cerisyi* clade cannot be unambiguously determined based on current data, as it is equally likely that the ancestral stock of *A. cerisyi* either dispersed northwards from Israel to Turkey and Europe, or from Europe to the south. My data also show no divergence between the Greek and Macedonian populations of *A. cerisyi* assigned to *A. c. huberi* compared those assigned to *A. c. ferdinandi*, and therefore do not support a separate status for these two taxa.

In order to provide a biogeographic hypothesis for the distribution of *Allancastria*, I compared the results of a previous divergence/vicariance and molecular clock analysis (Nazari, 2006) with the geological and tectonic history of the Mediterranean basin (after Steininger and Rõgl, 1996). The most parsimonious hypothesis for dispersal and vicariance for this genus would be that the ancestral *Allancastria* probably originated in the Iran-Anatolian plate in the early Miocene (21-19 MYA), and dispersed into the Afro-Arabian region upon extension of the Lower Fars Formation across the Mesopotamian Trough (~ 17 MYA). The separation of the Greece-Turkey-Yugoslavia landmass from Eurasia, and removal of the Fars Formation (16-15 MYA) would have subsequently isolated the three populations and gradually given rise to three ancestral species: *A. louristana* (Iran), *A. deyrollei* (Anatolia), and *A. cerisyi* (Afro-Arabia). During the middle Miocene (15-14 MYA), and upon formation of land bridges between the Middle East and Eurasia, ancestral *A. cerisyi* would have dispersed into Turkey and Greece. The Island of Crete [Kriti] later disconnected from the mainland around 11 MYA, giving rise to *A. cretica*. The populations of *A. caucasica* were isolated only in the Pliocene (3.5-3 MYA) upon flooding of the Mediterranean Sea which created water connections between the Mediterranean, Black and Caspian seas, leaving the Caucasus Mountains in the middle as islands.

Zerynthia. Based on a previous molecular clock analysis (Nazari, 2006), the two species of *Zerynthia* would have originated from a common ancestor around 16-18 MYA after a vicariance event (formation of the Mediterranean Basin) widely separated the ancestral range. The northern populations would have evolved into *Z. polyxena,* and the southern ones given rise to *Z. rumina.* For *Z. polyxena,* I was able to sample only a limited number of populations from Greece and Eastern Europe as well as Russia and the Ukraine. The overall genetic variation observed within the species was limited (0.6%), and the phylogeny is suggestive of dispersal from southern Europe towards the north and east, as the specimens from Ukraine and SW Russia form a clade in a crown node. Examination of Italian and other European populations might provide valuable insights into this perspective.

My phylogenetic reconstructions provide no meaningful distinction among the Spanish subspecies of *Z. rumina*, and specimens assigned to various populations of *Z. r. castiliana* seem to be paraphyletic with respect to other subspecies. The specimen from Islallana seems to be basal and shows a notable divergence compared to the rest of Spanish specimens. The status of other subspecies, including *Z. r. lusitanica* (Portugal), *Z. r. australis* (southern France), and *Z. r. tarrieri* (Moroccan Anti-Atlas) remains unknown.

The only African lineage of *Zerynthia rumina* studied here (*Z. r. africana* Stichel, 1907) was basal to all Spanish populations and showed a high degree of divergence (3.2%) from all other populations. This is as high as the divergence between most closely related species within the Parnassiinae (Fig. 3.3). However, morphology does not support such a distinction between the two populations: slight differences in wing markings noted previously in describing subspecies, and almost uniform genitalia across the entire range of *Z. rumina*, are not strong arguments for supporting species-level recognition. Further studies on the biology and early stages, as well as molecular work using more gene regions, might ultimately provide satisfactory evidence for the elevation of *Z. rumina africana* into a separate species.

The large gap and the basal position of the African population of *Z. rumina* suggest early dispersal of the ancestral stock of this species between Africa and Europe. Paleogeographical reconstructions show that the last known contact between Iberia and Africa occurred at the end of Miocene, with the formation of the Gibraltar arc which completely disconnected the Mediterranean sea from the Atlantic Ocean, causing extensive evaporation of the Mediterranean during the Messinian age (7-5.3

MYA) (also known as "the Messinian salinity crisis", Sanmartin, 2003). This event temporarily closed the water corridors between Africa and Iberian Peninsula, and permitted biotic exchange between the two continents (Krijgsman, 2002). The barrier was restored when Gibraltar re-opened by the start of the Pliocene (5 MYA). This short period of connection between the two continents probably accounts for vicariance between the North African and Iberian lineages of *Z. rumina*. This event has been suggested as a plausible explanation for vicariance between African/Iberian lineages of fishes in the Cyprinidae (Doadrio *et al.*, 1998) and beetles in Pachydeminae (Sanmartin, 2003).

Hypermnestra. The range of *H. helios* can be roughly divided into two regions: a) the Iranian plateau, an area delimited by the Zagros mountains in the west, Kopet-Dagh and the Lesser Caucasus mountains in the north, and the Pamir mountains in the east; and b) Central Asia, also known as the Turanian or Transcaspian region, extending from Turkmenistan to Kazakhstan, which in this case includes the remaining range of *H. helios*. The Central Asian populations of this species studied here (*H. h. helios* and *H. h. maxima*) demonstrate no genetic difference (0.0%), which supports their synonymy as suggested previously (Tschikolovets, 1998). The Iranian populations, however, are clearly distinct from Central Asian ones (2.6%) and show some variation as well (0.1%). My comparison of morphological characters between the two populations has shown that wing pattern elements are somewhat variable and not reliable for taxonomic work. The reduced number of teeth on the tip of the aedeagus in Iranian populations compared to those from Central Asia is suggestive of more substantive differences. However, this may still turn out to be a variable trait considering the limited number of specimens examined here. The Pakistani populations of *H. helios* hold the oldest available name for the Iranian Plateau group (*balucha* Moore, 1906) which should be used as the species name if this population is found to be part of the Iranian group. However, if these - and other populations from Afghanistan - are discovered to be part of the Central Asian group, the valid name to use for the Iranian group would be *H. h. bushirica* Bang-Haas, 1938, with *H. h. hyrcana* Sheljuzhko, 1956 as the subspecies from Northern Iran.

The separation of an ancestral *Hypermnestra* lineage from the last common ancestor of Parnassiini has been estimated to have taken place around the same time as India collided into Eurasia (65-42 MYA) (Nazari, 2006), which resulted in confinement of the ancestral *Hypermnestra* in the lowlands of Asia. Given the average divergence of the *COI* gene between *Parnassius* species studied here and *Hypermnestra* (16.56% corrected by GTR+G+I model), and given the average age of about 50 MY estimated for that event (sensu Nazari, 2006), the divergence of the two lineages within *Hypermnestra* (corrected to 3.35%) can be roughly estimated to have taken place around 10 MY ago. Formation of the Iranian plateau, which today stands as the main barrier between the two lineages, is also known to have begun about 10 MYA after the collision of the Arabian plate into Eurasia, resulting in the uplift of Zagros mountains in Miocene, and subsequently the Lesser Caucasus and Kopet-Dagh mountains in the early Pliocene (5 MYA) (Sanmartin, 2003). My results suggest that the two lineages of *H. helios* separated during or right after the formation of the Iranian plateau. This event has previously been suggested as the best explanation for the evolutionary divergences observed between Iranian and Central Asian populations of agamid Lizards (Macey *et al.* 1998) and in Pachydeminae beetles (Sanmartin, 2003).

Conclusion

My results show large gaps and higher than normal divergence rates within populations of *Archon apollinus* (2.7%), *H. helios* (2.6%) and *Z. rumina* (3.2 %), as well as more limited divergences between previously established species, i.e. *Archon apollinus* and *A. apollinaris* (1.9%), and *Allancastria cerisyi* and *A. caucasica* (1.0%). My attempt to find further morphological evidence to further evaluate such divergences was not successful. Although I believe that these high divergences present good indications of potential speciation events, I refrain from making any taxonomic conclusions before comprehensive morphological investigations are conducted, as well as examination of further molecular characters in more populations. I also observed several cases of substantial overlap in the range of uncorrected pairwise distances in mitochondrial *COI* gene between higher taxonomic categories in Parnassiinae butterflies.

I suggest that a revision of the genus *Archon*, based on further biological and molecular research, is needed for the synonymies proposed for infra-specific names within *Archon apollinus*, since the present molecular data support recognition of a significant distinction between the Israeli and Turkish subspecies while these have been previously proposed as synonyms (De Freina 1985). No decision can be made on the taxonomic status of the diverged populations within *H. helios* or *Z. rumina* without a thorough examination of specimens from a broader range, including populations of *H. helios* from Afghanistan and Pakistan, and *Z. rumina* from other localities in northern Africa as well as Europe.

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Table 3.1. Specimens examined, with their collection data and associated Genbank numbers.

	Species	subspecies	Locality	Specimen ID	GenBank
1	*Baronia brevicornis*		Mexico:Teacalco, btw Guerrero-Morelos, 07.1988	FS-a-167	AF170865
2	*Archon apollinus*	*apollinus*	Turkey: Bursa, Yaliciftilik, 11.03.2003	FS-b-2059	DQ383990
3	*Archon apollinus*	*apollinus*	Turkey: Mügla, Oludinez, 9.04.1999	FS-b-1868	DQ351031
4	*Archon apollinus*	*bellargus*	Israel: Emaus, 2 km NE Latrun, 10.03.2000	FS-b-2024	DQ383989
5	*Archon apollinaris*	*apollinaris*	Iran: Kermanshah, Rijab, 9.04.1998	FS-b-2025	DQ351032
6	*Archon apollinaris*	*apollinaris*	Turkey: 33 km Mardin-Diyarbakir, 15.04.1987	FS-b-2060	DQ383991
7	*Archon apollinaris*	*bostanchii*	Iran: Lorestan, Poledokhtar, 10.04.2003	FS-b-2063	DQ351033
8	*Luehdorfia japonica*	*japonica*	Japan: Shimizu Pass, Muikamichi, Niigata, 2.05.2002	FS-b-2033	DQ383987
9	*Luehdorfia japonica*	*japonica*	Japan: Kanazawa, Ishikawa, 20.02.1991	FS-a-335	AF170867
10	*Luehdorfia puziloi*	*puziloi*	Russia: Primoriye, Vladivostok	EZ-2-11	DQ351035
11	*Luehdorfia puziloi*	*lingjiangensis*	China: Liaoning, Nanzamu, 26.04.2005	FS-b-2118	DQ383988
12	*Luehdorfia taibai*	*taibai*	China: Shaanxi, Qinling, 06.2002	FS-b-2102	DQ351034
13	*Hypermnestra helios*	*helios*	SE Kazakhstan: Ili Rive, Bakanas village, 1-15.05.1998	FS-b-1597	DQ351025
14	*Hypermnestra helios*	*helios*	SE Kazakhstan: Ili Rive, Bakanas village, 1-3.05.2002	FS-b-2071	DQ383986
15	*Hypermnestra helios*	*maxima*	Tajikistan: Kurgan-Tube, 20 km S Dzhilikul, 10.05.2000	FS-b-2070	DQ383985
16	*Hypermnestra helios*	*maxima*	Uzbekstan: Fergana Valley, Komsomolabad, 20.05.2002	FS-b-2069	DQ383984
17	*Hypermnestra helios*	*hyrcana*	Iran:Tehran, Karaj, Jaroo Mt., 11.05.2002	FS-b-2067	DQ383982
18	*Hypermnestra helios*	*bushirica*	Iran:Hormozgan, 40 km N Bandarabbas, 16.03.1998	FS-b-2068	DQ383983
19	*Parnassius phoebus*		Canada: AB: Plateau Mt., 08.1986	FS-a-8	AF170872
20	*Parnassius schultei*		China: Tibet, Trans-himalaya, Karola Pass, 22-28.06.1994	FS-b-1978	DQ351026
21	*Parnassius tenedius*		Kirghizstan: Altai Mts., Aktash village, 16.05.1997	FS-b-1784	DQ351027
22	*Parnassius delphius*		Kirghizstan: Tian-Shan, Naryntoo Mts., 1-10.07.1996	FS-b-1775	DQ351028
23	*Parnassius autocrator*		Tajikistan: E. Pamir, Muzkoi Mts., W Morgav village, 08.2000	FS-b-1983	DQ351029
24	*Parnassius simonius*		Kirghizstan: Transalai Mts., 1-20.07.1998	FS-b-1777	DQ351030
25	*Parnassius clodius*		USA: WA: Okanagen Co., Chinook Pass, 7.03.1986	FS-a-375	AF170871
26	*Bhutanitis mansfieldi*	*mansfieldi*	China: Sichuan, E'mei Mt., 07.2000	FS-b-1589	DQ351036
27	*Bhutanitis mansfieldi*	*mansfieldi*	China: Sichuan, E'mei Mt., 07.2001	FS-b-2041	DQ383994
28	*Bhutanitis thaidiana*	*thaidiana*	China: Sichuan, Daba Mt., 07.2000	FS-b-1591	DQ351037
29	*Bhutanitis thaidiana*	*thaidiana*	China: Sichuan, Daba Mt., 06.2002	FS-b-2043	DQ383995
30	*Bhutanitisi lidderdali*	*lidderdali*	China: Yunnan, Dongchuan County, 10.2002	FS-b-2044	DQ351038
31	*Sericinus montela*	*montela*	Japan: Tanashi, near Tokyo, emg. 4.04.1991	FS-a-399	AF170867
32	*Sericinus montela*	*koreanus*	Korea: Near Seoul	FS-b-2028	DQ383992
33	*Sericinus montela*	*amurensis*	Russia: Primoriye, 5-9.08.1998	FS-b-2090	DQ383993
34	*Zerynthia polyxena*	*albanica*	Kosovo: Prizren, 8-9.05.2003	FS-b-2072	DQ384005
35	*Zerynthia polyxena*	*bosniensis*	Serbia: Petlovo Brdo, 27.05.2003	FS-b-2073	DQ384006
36	*Zerynthia polyxena*	*macedonica*	Greece: Florina, 9-10.05.2003	FS-b-2066	DQ384004
37	*Zerynthia polyxena*	*bryki*	Montenegro: Lovten, Crna Gora, 29.05.2003	FS-b-2045	DQ384003
38	*Zerynthia polyxena*	*petri*	Ukraine: Zaporozhje env., 25.05.2002	FS-b-2075	DQ384008
39	*Zerynthia polyxena*	*petri*	Russia: District of Stravropol, 12.04.2002	FS-b-2074	DQ384007
40	*Zerynthia polyxena*	*petri*	Russia: District of Voronezh, 1-5.05.1998	FS-b-1596	DQ351039
41	*Zerynthia rumina*	*africana*	Morocco: Near Casablanca, 06.2002	FS-b-2040	DQ383996
42	*Zerynthia rumina*	*castiliana*	Spain: Islallana, La Rioja, 10.05.2003	FS-b-2053	DQ383997
43	*Zerynthia rumina*	*castiliana*	Spain: N. Burgos, Nidaguila, 20.05.2003	FS-b-2054	DQ383998
44	*Zerynthia rumina*	*castiliana*	Spain: N. Burgos, Temino, 29.04.2003	FS-b-2055	DQ383999
45	*Zerynthia rumina*	*castiliana*	Spain: S. Burgos, Hortiguela, 6.04.1995	FS-b-2056	DQ384000
46	*Zerynthia rumina*	*cantabricae*	Spain: Cantabria, Aldea de Ebro, Pozazal, 15.05.2003	FS-b-2052	DQ384002
47	*Zerynthia rumina*	*rumina*	Spain: Malaga (1), emg. 5.11.1989	FS-a-88	AF170870
48	*Zerynthia rumina*	*rumina*	Spain: Malaga (2), Ronda Prov., Gaucin, 22.03.2002	FS-b-2057	DQ384001
49	*Allancastria louristana*	*louristana*	Iran: Lorestan, Malavi, 04.04.1999	FS-b-2037	DQ351040
50	*Allancastria cretica*	*cretica*	Greece: Kriti Island, Lassithi, 4.05.2003	FS-b-2038	DQ351041
51	*Allancastria caucasica*	*caucasica*	Turkey: Bolu Prov., Bolu Daglari, 21.04.2001	FS-b-2046	DQ351042
52	*Allancastria cerisyi*	*cerisyi*	Turkey: Davultlar, 8.04.2002	FS-b-2077	DQ384016
53	*Allancastria cerisyi*	*speciosa*	Israel: Karen Hacarmel, Carmel Mt., 7.04.2000	FS-b-2034	DQ384014
54	*Allancastria cerisyi*	*cypria*	Greece: Cyprus, Paphos Prov., Polis, 3-9.05.1999	FS-b-2076	DQ384015
55	*Allancastria cerisyi*	*huberi*	Greece: Florina, 9-10.05.2003	FS-b-2078	DQ384017
56	*Allancastria cerisyi*	*huberi*	Macedonia: Bitola, 12.05.2003	FS-b-2079	DQ384018
57	*Allancastria cerisyi*	*ferdinandi*	Macedonia: Katlanovo, 5.05.2003	FS-b-2080	DQ384019
58	*Allancastria cerisyi*	*ferdinandi*	Kosovo: Kachanik, 7.05.2003	FS-b-2081	DQ384020
59	*Allancastria cerisyi*	*ferdinandi*	Macedonia/Bulgaria/Greece border: Belasica Mt., 15.05.2003	FS-b-2082	DQ384021
60	*Allancastria cerisyi*	ssp.	Greece: Thessaloniki, 1990	FS-a-342	AF170869
61	*Allancastria deyrollei*	*deyrollei*	Turkey: Yozgat, vic. Yerkoy, 2003	FS-b-2088	DQ384012
62	*Allancastria deyrollei*	*deyrollei*	Turkey: Van, W Gevas, Kushunkiran Pass, 2003	FS-b-2087	DQ384011
63	*Allancastria deyrollei*	*eisneri*	Iran: Kermanshah, Rijab, 5.04.1999	FS-b-2036	DQ384009
64	*Allancastria deyrollei*	*eisneri*	Iran: W Azarbaijan, Takab, 23.05.2003	FS-b-2068	DQ351043
65	*Allancastria deyrollei*	*eisneri*	Iran: W Azarbaijan, E Marand Rd. Boukan, 2003	FS-b-2089	DQ384013
66	*Allancastria deyrollei*	*eisneri*	Israel: Canada Park, Latrun, 15.06.2003	FS-b-2083	DQ384010

Table 3.2. Primers used in this study.

Location*	Name	Source	F/R	Sequence (5'→3')
1751	RonIII	Caterino & Sperling 1999	F	GGA GCA CCT GAC ATA GCT TTC CC
2183	Jerry	Simon *et al.* 1994	F	CAA CAT TTA TTT TGA TTT TTT GG
2329	K525	Simon *et al.* 1994	R	ACT GTA AAT ATA TGA TGA GCT CA
2329	K525.2	Caterino *et al.* 2001	R	ACA GTA AAT ATA TGA TGA GCT CA
2329	K525.4	Caterino *et al.* 2001	R	ACT GTG AAT ATG TGA TGG GCT CA
2495	BrianXXI	Caterino *et al.* 2001	F	CCT CAA TTT TAT GAA GAT TAG G
2658	Mila7	Caterino & Sperling 1999	R	GAA AGT CCA GTA AAT AAA GG
2837	George	Bogdanowicz *et al.* 1993	F	ATA CCT CGA CGT TAT TCA GA
3014	Pat	Simon *et al.* 1994	R	TCC AAT GCA CTA ATC TGC CAT ATT A
3014	PatII	Sperling *et al.* 1996	R	TCC ATT ACA TAT AAT CTG CCA TAT TAG

*** Positions relative to *Drosophila yakuba* mtDNA (Clary and Wolstenholme, 1985).**

Table 3.3. Average uncorrected pairwise distances between species in Parnassiinae based on 825 bp of *COI* . Bold values in boxes are cases of relatively low genetic diversity between recognized pairs of species, and those highlighted are the ones noted through this study as being high for variation within species (*H. helios, A. apollinus, Z. rumina*). N indicates number of specimens examined in this study, and numbers on the diagonal represent mean genetic variation among specimens within terminal taxa.

			N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Parnassiini	1	*P. phoebus*	1	-																											
	2	*P. schultei*	1	5.8	-																										
	3	*P. tenedius*	1	7.8	6.3	-																									
	4	*P. delphius*	1	5.8	4.7	5.7	-																								
	5	*P. autocrator*	1	6.8	5.1	7.0	5.0	-																							
	6	*P. simonius*	1	6.1	4.6	6.5	5.5	5.8	-																						
	7	*P. clodius*	1	5.9	4.6	7.3	5.8	6.7	4.6	-																					
	8	*H. helios* C Asia	4	9.6	8.6	8.6	9.0	9.1	9.5	8.7	0.0																				
	9	*H. helios* Iran	2	8.6	8.8	8.8	8.9	9.2	9.8	8.7	[illegible]	0.1																			
Luehdorfiini	10	*L. japonica*	2	9.9	8.6	10.2	9.7	9.8	9.9	8.8	11.8	11.5	0.2																		
	11	*L. puziloi*	2	9.8	8.5	9.5	9.2	10.1	10.5	8.9	11.2	11.6	5.5	0.1																	
	12	*L. taiba*	1	9.2	7.4	9.1	8.6	8.7	8.8	7.6	10.8	11.2	4.8	4.4	-																
	13	*A. apollinus* Turkey	2	10.2	9.5	10.5	9.9	10.4	10.7	10.4	10.5	10.6	7.8	8.7	8.2	0.4															
	14	*A. apollinus* Israel	1	10.4	9.7	10.7	9.7	11.2	10.8	10.8	9.9	10.4	8.4	9.3	9.0	[illegible]	-														
	15	*A. ap. apollinaris*	2	10.1	9.2	9.8	9.7	10.6	10.3	10.1	10.2	10.4	7.8	8.2	8.1	**1.9**	3.0	0.0													
	16	*A. ap. bostanchii*	1	10.4	9.2	9.8	9.7	10.6	10.3	10.1	10.4	10.4	7.9	8.9	8.5	2.3	3.2	2.1	-												
Zerynthiini	17	*S. montela*	3	9.3	9.1	9.8	9.7	10.7	10.1	9.3	10.5	11.0	9.7	9.9	9.5	9.1	9.1	8.0	9.1	0.0											
	18	*B. mansfieldi*	2	12.1	10.8	11.0	11.5	11.9	11.8	10.8	11.9	12.2	10.7	11.1	10.8	9.0	9.3	8.7	8.2	10.4	0.0										
	19	*B. thaidiana*	2	11.6	11.3	12.4	11.5	12.8	12.1	11.3	11.9	12.4	11.5	11.2	11.0	9.4	10.4	9.2	10.0	10.0	7.6	0.1									
	20	*B. lidderdali*	1	12.7	11.8	11.5	11.0	12.1	12.1	12.0	12.7	12.7	12.0	12.3	11.4	10.1	10.2	9.3	9.2	10.5	7.3	7.7	-								
	21	*Z. rumina* N Africa	1	11.7	11.0	10.9	11.5	12.0	11.7	11.0	12.4	12.7	11.0	10.9	10.0	9.8	10.4	9.8	9.7	10.4	10.2	10.7	10.9	-							
	22	*Z. rumina* Spain	7	12.8	11.6	11.7	12.3	12.2	12.2	11.6	12.5	12.8	11.9	12.1	11.2	10.4	11.0	10.4	10.1	10.8	10.7	11.1	11.0	[illegible]	1.1						
	23	*Z. polyxena*	7	10.9	10.5	11.4	11.2	11.3	11.9	11.4	12.3	12.9	10.7	10.9	9.9	9.1	10.0	8.8	8.4	9.1	9.4	10.7	10.3	6.1	6.4	0.6					
	24	*A. louristana*	1	10.5	10.2	11.3	11.0	11.6	11.2	10.5	10.8	11.5	10.4	10.7	9.9	8.1	8.8	8.0	8.4	8.8	9.9	10.7	10.4	7.9	8.7	7.0	-				
	25	*A. deyrollei*	6	10.7	10.3	11.0	11.0	11.2	11.3	10.6	11.6	11.7	10.6	10.7	9.9	8.4	9.2	8.3	8.7	8.7	9.6	11.1	10.2	8.3	8.8	7.7	3.9	0.8			
	26	*A. cretica*	1	10.9	10.4	11.2	10.9	11.6	11.4	11.0	11.6	11.8	10.7	11.0	10.2	9.0	9.2	8.4	9.0	8.7	9.5	10.5	10.1	8.6	9.5	7.8	4.5	3.0	-		
	27	*A. caucasica*	1	10.9	10.9	10.9	11.6	12.1	11.9	11.5	11.4	11.8	11.3	11.0	10.4	9.3	9.6	8.7	9.3	8.7	10.3	11.2	10.5	9.2	9.8	8.2	4.6	3.4	2.3	-	
	28	*A. cerisyi*	9	11.1	10.7	11.1	11.5	11.9	11.4	10.9	11.3	11.6	11.0	10.9	10.3	9.4	9.4	8.8	9.2	9.1	10.1	10.9	10.0	9.2	9.5	8.0	4.3	3.3	2.3	**1.0**	0.4

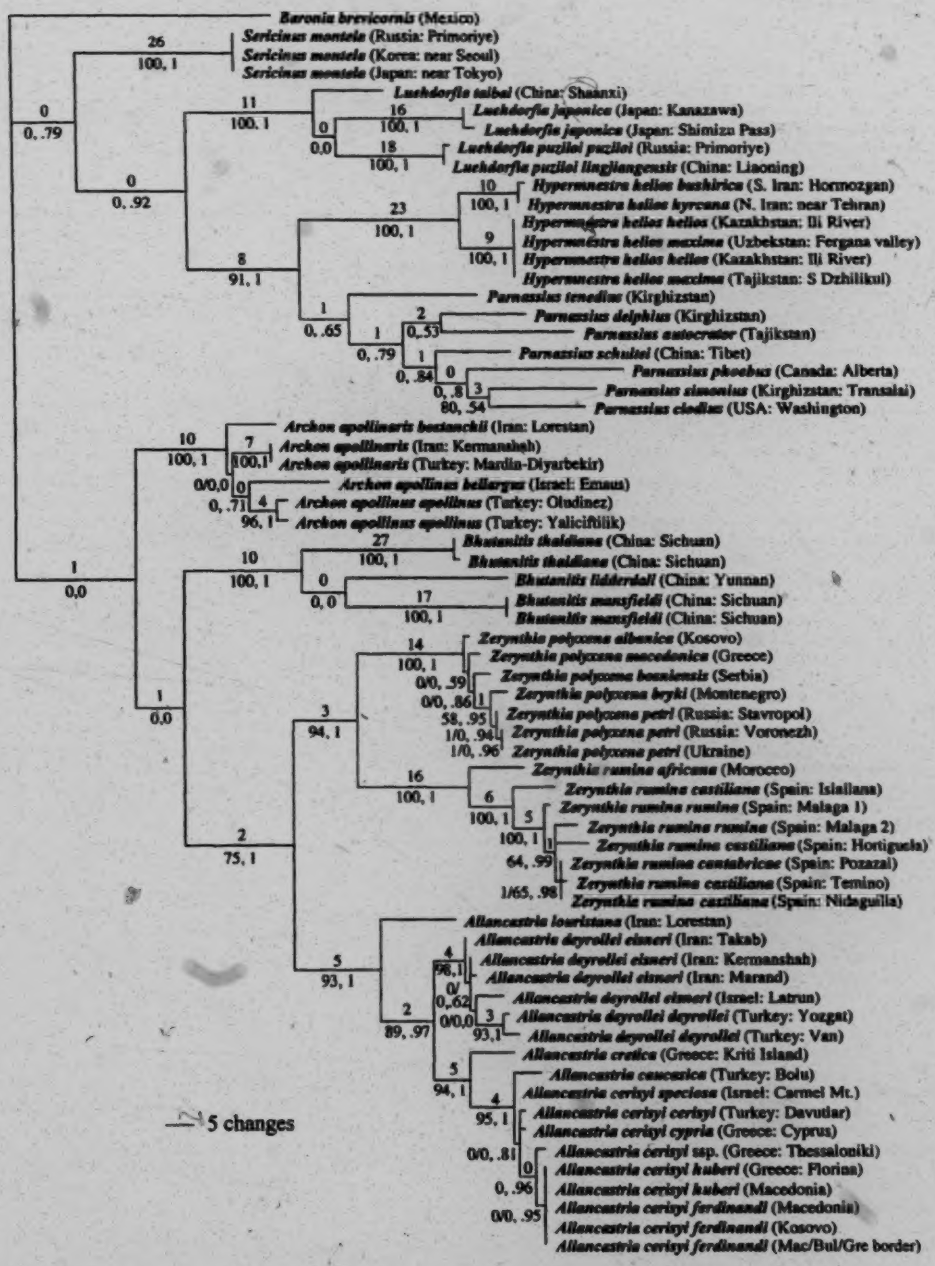
Baronia brevicornis (Mexico)
26
100, 1
Sericinus montela (Russia: Primoriye)
Sericinus montela (Korea: near Seoul)
Sericinus montela (Japan: near Tokyo)
0
0, .79
Luehdorfia taibai (China: Shaanxi)
11
100, 1
16
100, 1
0
0,0
Luehdorfia japonica (Japan: Kanazawa)
Luehdorfia japonica (Japan: Shimizu Pass)
18
100, 1
Luehdorfia puziloi puziloi (Russia: Primoriye)
Luehdorfia puziloi lingjiangensis (China: Liaoning)
0
0, .92
10
100, 1
Hypermnestra helios bushirica (S. Iran: Hormozgan)
Hypermnestra helios hyrcana (N. Iran: near Tehran)
23
100, 1
Hypermnestra helios helios (Kazakhstan: Ili River)
9
100, 1
Hypermnestra helios maxima (Uzbekstan: Fergana valley)
Hypermnestra helios helios (Kazakhstan: Ili River)
Hypermnestra helios maxima (Tajikstan: S Dzhilikul)
8
91, 1
Parnassius tenedius (Kirghizstan)
1
0, .65
2
0,.53
Parnassius delphius (Kirghizstan)
Parnassius autocrator (Tajikstan)
1
0, .79
1
0, .84
Parnassius schulzei (China: Tibet)
0
0, .8
Parnassius phoebus (Canada: Alberta)
3
80, .54
Parnassius simonius (Kirghizstan: Transalai)
Parnassius clodius (USA: Washington)
Archon apollinaris bostanchii (Iran: Lorestan)
10
100, 1
7
100,1
Archon apollinaris (Iran: Kermanshah)
Archon apollinaris (Turkey: Mardin-Diyarbekir)
0/0,0
0
0, .71
Archon apollinus bellargus (Israel: Emaus)
4
96, 1
Archon apollinus apollinus (Turkey: Oludinez)
Archon apollinus apollinus (Turkey: Yaliciftilik)
1
0,0
27
100, 1
Bhutanitis thaidiana (China: Sichuan)
Bhutanitis thaidiana (China: Sichuan)
10
100, 1
0
0, 0
Bhutanitis lidderdalii (China: Yunnan)
17
100, 1
Bhutanitis mansfieldi (China: Sichuan)
Bhutanitis mansfieldi (China: Sichuan)
1
0,0
14
100, 1
Zerynthia polyxena albanica (Kosovo)
Zerynthia polyxena macedonica (Greece)
0/0, .59
Zerynthia polyxena bosniensis (Serbia)
0/0, .86
1
Zerynthia polyxena bryki (Montenegro)
58, .95
Zerynthia polyxena petri (Russia: Stavropol)
1/0, .94
Zerynthia polyxena petri (Russia: Voronezh)
1/0, .96
Zerynthia polyxena petri (Ukraine)
3
94, 1
16
100, 1
Zerynthia rumina africana (Morocco)
6
100, 1
Zerynthia rumina castiliana (Spain: Islallana)
5
100, 1
Zerynthia rumina rumina (Spain: Malaga 1)
1
Zerynthia rumina rumina (Spain: Malaga 2)
Zerynthia rumina castiliana (Spain: Hortiguela)
64, .99
Zerynthia rumina cantabricae (Spain: Pozazal)
Zerynthia rumina castiliana (Spain: Temino)
1/65, .98
Zerynthia rumina castiliana (Spain: Nidaguilla)
2
75, 1
Allancastria louristana (Iran: Lorestan)
5
93, 1
4
98,1
Allancastria deyrollei eisneri (Iran: Takab)
Allancastria deyrollei eisneri (Iran: Kermanshah)
Allancastria deyrollei eisneri (Iran: Marand)
0/
0,.62
Allancastria deyrollei eisneri (Israel: Latrun)
2
89, .97
0/0,0
3
93,1
Allancastria deyrollei deyrollei (Turkey: Yozgat)
Allancastria deyrollei deyrollei (Turkey: Van)
5
94, 1
Allancastria cretica (Greece: Kriti Island)
Allancastria caucasica (Turkey: Bolu)
4
95, 1
Allancastria cerisyi speciosa (Israel: Carmel Mt.)
Allancastria cerisyi cerisyi (Turkey: Davutlar)
Allancastria cerisyi cypria (Greece: Cyprus)
0/0, .81
Allancastria cerisyi ssp. (Greece: Thessaloniki)
0
0, .96
Allancastria cerisyi huberi (Greece: Florina)
Allancastria cerisyi huberi (Macedonia)
0/0, .95
Allancastria cerisyi ferdinandi (Macedonia)
Allancastria cerisyi ferdinandi (Kosovo)
Allancastria cerisyi ferdinandi (Mac/Bul/Gre border)
5 changes

Fig. 3.1. Maximum likelihood phylogeny of Parnassiinae (TL= 906, CI= 0.434, RI= 0.832) inferred from 825 bp of *COI*. Numbers above the branches are Bremer support values; numbers below the branches are bootstrap values from parsimony analysis followed by Bayesian posterior probabilities from Bayesian analysis.

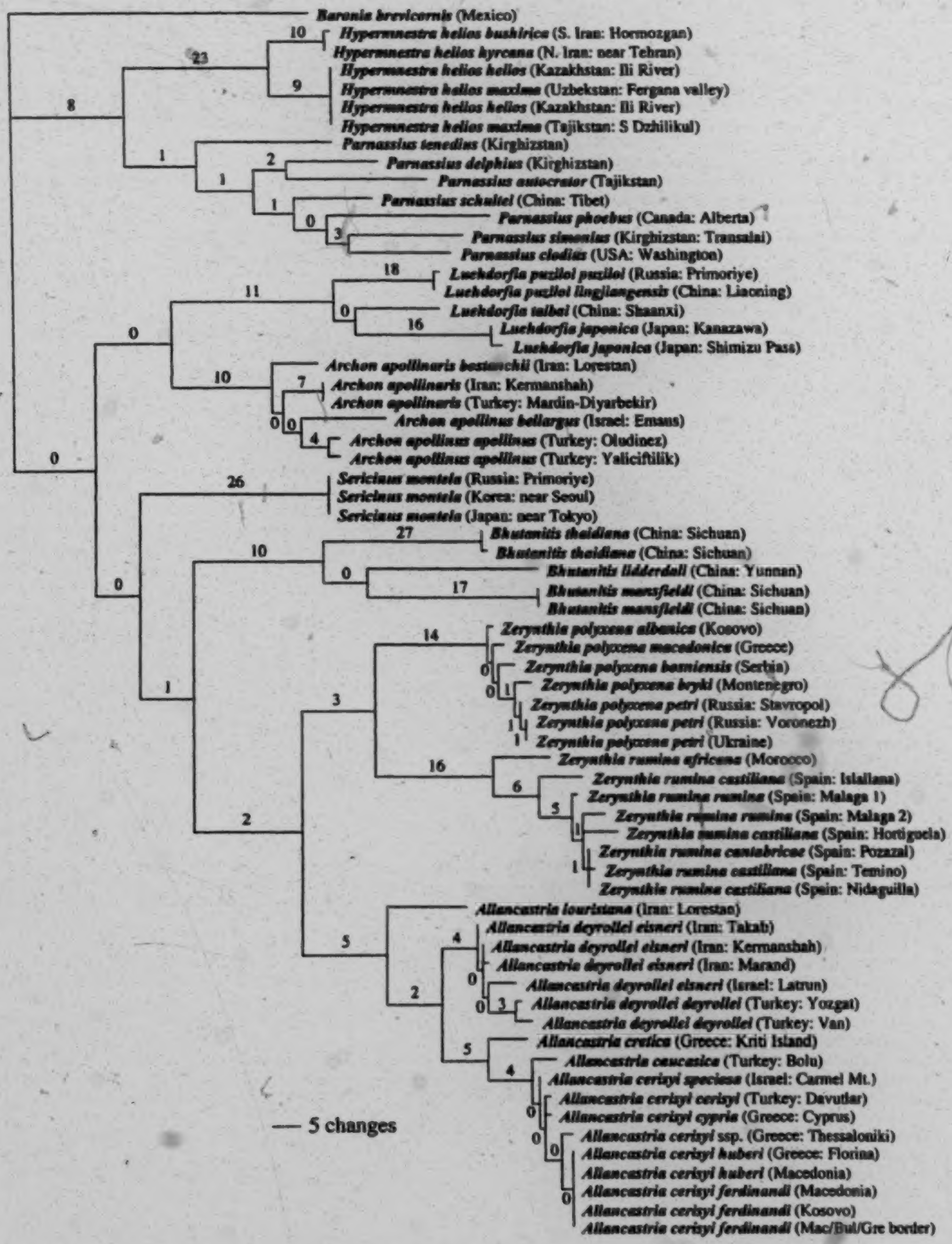
Baronia brevicornis (Mexico)
10
Hypermnestra helios bushirica (S. Iran: Hormozgan)
23
Hypermnestra helios hyrcana (N. Iran: near Tehran)
Hypermnestra helios helios (Kazakhstan: Ili River)
9
Hypermnestra helios maxima (Uzbekstan: Fergana valley)
8
Hypermnestra helios helios (Kazakhstan: Ili River)
Hypermnestra helios maxima (Tajikstan: S Dzhilikul)
Parnassius tenedius (Kirghizstan)
1
2
Parnassius delphius (Kirghizstan)
1
Parnassius autocrator (Tajikstan)
1
Parnassius schultei (China: Tibet)
0
Parnassius phoebus (Canada: Alberta)
3
Parnassius simonius (Kirghizstan: Transalai)
Parnassius clodius (USA: Washington)
18
Luehdorfia puziloi puziloi (Russia: Primoriye)
11
Luehdorfia puziloi lingjiangensis (China: Liaoning)
0
Luehdorfia taibai (China: Shaanxi)
16
Luehdorfia japonica (Japan: Kanazawa)
0
Luehdorfia japonica (Japan: Shimizu Pass)
Archon apollinaris bostanchii (Iran: Lorestan)
10
7
Archon apollinaris (Iran: Kermanshah)
Archon apollinaris (Turkey: Mardin-Diyarbekir)
0
0
Archon apollinus bellargus (Israel: Emaus)
4
Archon apollinus apollinus (Turkey: Oludinez)
0
Archon apollinus apollinus (Turkey: Yaliciftilik)
26
Sericinus montela (Russia: Primoriye)
Sericinus montela (Korea: near Seoul)
Sericinus montela (Japan: near Tokyo)
27
Bhutanitis thaidiana (China: Sichuan)
10
Bhutanitis thaidiana (China: Sichuan)
0
Bhutanitis lidderdali (China: Yunnan)
17
0
Bhutanitis mansfieldi (China: Sichuan)
Bhutanitis mansfieldi (China: Sichuan)
Zerynthia polyxena albanica (Kosovo)
14
Zerynthia polyxena macedonica (Greece)
0
Zerynthia polyxena bosniensis (Serbia)
1
0
1
Zerynthia polyxena bryki (Montenegro)
3
Zerynthia polyxena petri (Russia: Stavropol)
1
Zerynthia polyxena petri (Russia: Voronezh)
1
Zerynthia polyxena petri (Ukraine)
Zerynthia rumina africana (Morocco)
16
Zerynthia rumina castiliana (Spain: Islallana)
6
Zerynthia rumina rumina (Spain: Malaga 1)
5
Zerynthia rumina rumina (Spain: Malaga 2)
2
1
Zerynthia rumina castiliana (Spain: Hortigoela)
Zerynthia rumina cantabricae (Spain: Pozazal)
1
Zerynthia rumina castiliana (Spain: Temino)
Zerynthia rumina castiliana (Spain: Nidaguilla)
Allancastria louristana (Iran: Lorestan)
Allancastria deyrollei eisneri (Iran: Takab)
4
5
Allancastria deyrollei eisneri (Iran: Kermanshah)
Allancastria deyrollei eisneri (Iran: Marand)
0
Allancastria deyrollei eisneri (Israel: Latrun)
2
0
3
Allancastria deyrollei deyrollei (Turkey: Yozgat)
Allancastria deyrollei deyrollei (Turkey: Van)
Allancastria cretica (Greece: Kriti Island)
5
Allancastria caucasica (Turkey: Bolu)
4
Allancastria cerisyi speciosa (Israel: Carmel Mt.)
Allancastria cerisyi cerisyi (Turkey: Davutlar)
0
Allancastria cerisyi cypria (Greece: Cyprus)
5 changes
0
Allancastria cerisyi ssp. (Greece: Thessaloniki)
0
Allancastria cerisyi huberi (Greece: Florina)
Allancastria cerisyi huberi (Macedonia)
Allancastria cerisyi ferdinandi (Macedonia)
0
Allancastria cerisyi ferdinandi (Kosovo)
Allancastria cerisyi ferdinandi (Mac/Bul/Gre border)

Fig. 3.2. Maximum likelihood tree obtained with internal nodes constrained to reflect the higher-level phylogeny proposed by Nazari, 2006 (TL= 907, CI= 0.433, RI= 0.831). Numbers indicate Bremer support.

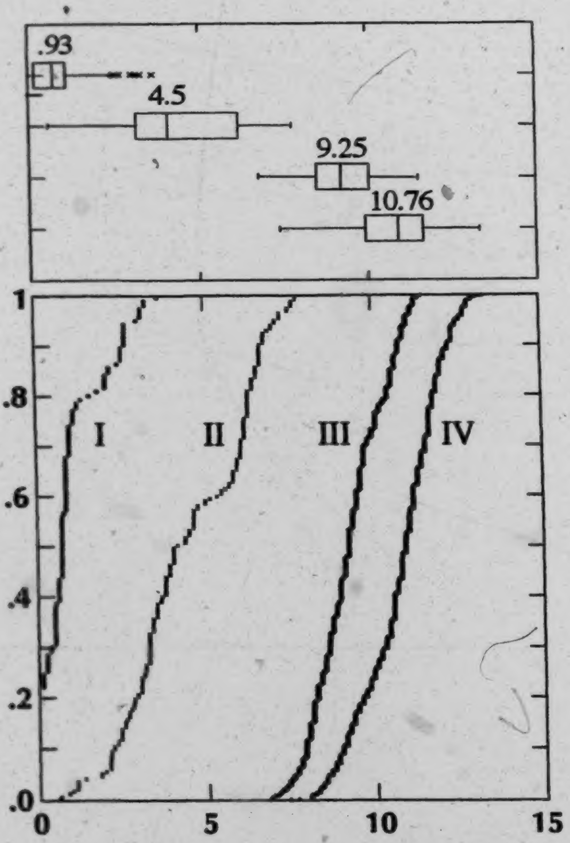

Fig 3.3. Cumulative pairwise differences within species (I), between species within genera (II), between genera within tribes (III) and between tribes (IV) of Parnassiinae species and specimens examined in this study. Box plots above the graph summarize the variation. The line in each box plot marks the median of the values; the length of the box shows the range within which the central 50% of the values fall; and whiskers show the range of values that fall within the inner fences (see SYSTAT manual for details). The outliers in the first category are those discussed in this paper as being potentially distinct species, since they fall within the range of genetic differentiation commonly found between established species.

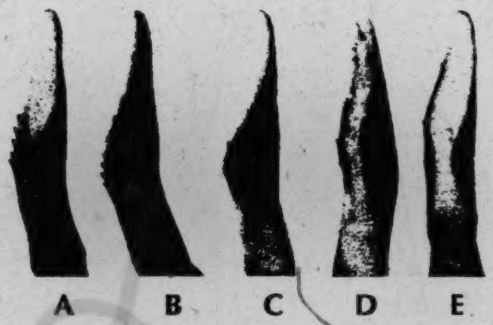

Fig 3.4. Tip of the aeadeagus in specimens from various populations of *Hypermnestra helios*. A) Uzbekistan, B) Tajikistan, C) Turkmenistan, D) N. Iran, E) S. Iran.

Chapter 4: Discussion and conclusion

The initial idea behind this masters program was to determine the higher phylogeny of the swallowtail subfamily Parnassiinae (Lepidoptera, Papilionidae), and to find new evidence from DNA sequences that could potentially resolve long-standing controversies regarding the classification of the subfamily (Ford, 1944; Munroe, 1961; Ackery, 1975; Hiura, 1980; Hancock, 1983; Stekolnikov and Kuznetsov, 2003). In the course of the program I looked at sequences from several gene regions as well as morphology. I also tried to find out how well genes perform in providing resolution for phylogenetic analyses, whether all genes perform equally well, or some provide better resolution than the others: which gene sequences should be looked at for resolving higher relationships, and which are better for population level studies? Also, how well are the molecular results correlated with morphology?

In the first chapter of this thesis I present a synopsis of classification of the subfamily Parnassiinae. This is a small group of mainly Old World butterflies with an east-west distribution in the Palaearctic (Fig. 2-1.). The classification of the group has been debated extensively over the past century (Bryk 1934, 1935; Talbot, 1939; Ford, 1944; Clench, 1955; Ehrlich, 1958; Munroe, 1961; Eisner, 1974; Higgins, 1975; Ackery, 1975; Igarashi, 1984; Chunsheng, 2001; Hemming, 1960), and even the monophyly of the group has been questioned (Hiura, 1980; Häuser, 1993). Throughout these studies, several hypotheses have been presented on the higher classification of the subfamily, but there has been an overall consensus on the presence of two tribes: Parnasiini (*Parnassius* + *Hypermnestra* + *Archon*), and

Zerynthiini (*Zerynthi* + *Allancastria* + *Sericinus* + *Bhutanitis* + *Luehdorfia*). Some morphological studies had suggested that *Archon* and *Luehdofia* are different from other members of their respective group (De Freina, 1985; Koçak, 1989; Stekolnikov and Korshunov, 2003), but these two were never thought to be associated before recent molecular studies (Omoto *et al.* 2004; Katoh *et al.* 2005). Furthermore, the phylogenetic position of *Hypermnestra* was uncertain, as it was thought to be either the closest sister to *Parnassius* (Ford, 1944; Ackery, 1975), or basal to all other genera in the subfamily (Hiura, 1980; Hesselbarth *et al.*, 1995).

I discuss these classifications in detail in Chapter 2. For this chapter I sequenced seven genes, including five mitochondrial (*COI, COII, ND5, ND1*, and *16S*) and two nuclear (*EF-1a* and *wg*), and used them together with many previously published sequences available from GenBank. Overall, more than 56% of the sequences were new (not including the *EF-1a* sequences that were extended for the 245 bp at 5' end; see table 2.1). My combined molecular dataset comprised 5775 nucleotide basepairs. I also looked at 236 informative morphological characters mostly selected from previously published studies. I performed analyses on partitioned and combined data, under maximum parsimony, maximum likelihood, and Bayesian criteria. I found that nuclear genes often perform better in providing resolution for higher classification, and that there are substantial differences in performance of mitochondrial genes. While the results of my morphological analysis were to some extent congruent with previous hypotheses, my molecular data presented a new arrangement of the higher relationships among the genera within the subfamily Parnassiinae, reflecting *three* groups of genera, namely Parnassiini (*Parnassius*+

Hypermnestra), Luehdorfiini **stat. nov.** (*Luehdorfia+Archon*), and Zerynthiini (*Zerynthia+Allancastria+ Sericinus+Bhutanitis*). The name Luehdorfiini had been erected previously based on genitalic characters (Stekolnikov and Kuznetsov, 2003), although it included only genus *Luehdorfia* and its position on the phylogeny was presented as basal to the subfamily. The association of *Archon* and *Luehdorfia* had also been previously reported based on single gene regions of smaller lengths (Omoto *et al.* 2004; Katoh *et al.* 2005), but it was regarded only as a phylogenetic curiosity which needed further investigation. In my study, despite the weak support obtained for the monophyly of the subfamily, most nuclear and mitochondrial genes presented these three groups, and in combination provided strong support for this hypothesis.

The evidence from the magnitude of genetic divergences of the three groups from each other was somewhat ambiguous and hence not comparable with those of the tribes in the closest sister subfamily, Papilioninae. Nonetheless, I considered the clear monophyly and distinct gaps of these three groups in presenting the new higher classification scheme for the subfamily. All of these groups were clearly monophyletic with high support (Bremer, bootstrap and Bayesian posterior probabilities), and all presented distinct gaps from each other in most of the phylogenies generated based on individual gene partitions as well as combined data.

To find out whether the new classification was congruent with the present distribution pattern of genera and species of Parnassiinae, I conducted a dispersal/vicariance (DIVA) analysis, and also tried dating the nodes on my phylogeny using molecular clock techniques with previously published divergence dates for Papilionidae. Results showed that the last common ancestor of the subfamily probably inhabited central Asia and China, and that the initial diversification of the subfamily probably began around 50 MY ago. This corresponded roughly to the time when the Indian plate collided with Eurasia, creating the Himalayas and splitting the range of the last common ancestor of the subfamily. A series of vicariance and dispersal events (26 in the most parsimonious reconstruction) through the Miocene shaped the current distribution pattern of the subfamily.

In chapter 3, I examined sequences from the *cytochrome oxidase I* (*COI*) mitochondrial gene to find out whether there is genetic basis for some of the infra-specific names described for the butterflies of the subfamily. The *COI* gene has been previously demonstrated to be helpful in providing resolution for phylogenetic studies within genera in Lepidoptera (Caterino *et al.* 2001, Vila and Bjorklund, 2004; Zakharov *et al.* 2004; Matsumura *et al.*, 2005; Silva-Brandao *et al.*, 2005). I sequenced many specimens from various subspecies of Parnassiinae from Europe to Central Asia. This study had two main outcomes: 1) some subspecies of certain species in the subfamily (namely *Archon apollinus, Zerynthia rumina,* and *Hypermnestra helios*) show a considerable divergence, which is comparable to the divergence between many sister species in the subfamily, and 2) some of the previously demonstrated species within the group (namely *Archon apollinaris* and *Archon apollinus*) are genetically much less diverged from each other. The results of this study also showed no genetic differentiation among many subspecies, which suggests their synonymy.

In conclusion, upon looking at many mitochondrial and nuclear gene regions and morphology of nearly every species in the subfamily through 3 years of extensive lab work, I found out that finding answers to questions regarding the evolutionary history of organisms, particularly butterflies, might not be as easy as it seems. I learned that different genes present different phylogenetic signals depending on a variety of factors, including saturation, and that they are not always congruent with what morphology might suggest. There might be substantial variation based on the type of analytical approach, whether simple distance methods (neighbor joining), classical parsimony, or more computationally intensive likelihood (including Bayesian) methods. Also, there is virtually no limit to the data that can be added to a dataset, and hence the choice of which gene region or morphological characters one should look at is crucial in saving cost and time, and obtaining results.

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Appendix 1: List of morphological characters investigated in this study.

Wherever possible, characters have been evaluated by V. Nazari; in all other cases the source of character coding is indicated. For a list of specimens examined, see Appendix 2.

a) Adult head:

1- **Distance between antennae at base:** 0 = at least twice the width of scape apart, 1 = less than twice the width of scape apart.

Ehrlich (1958), Munroe (1961) and De Jong *et al.* (1996, character 67). Antennae in Hesperiidae are far apart at base; in all other butterflies they are proximate near base.

2- **Antennal length:** 0 = short (≤ 1/4 length of forewing), 1 = long (>1/4 length of forewing).

Considered a useful character by several previous workers (e.g. Ford, 1944; Ehrlich, 1958). In this study, the antennal length is measured as the ratio of length of antennae relative to the length of forewing (base to apex) after Saigusa and Lee (1982).

3- **Antennal covering:** 0 = scaled, 1 = naked (except for the basal segments).

Ford (1944), Ehrlich (1958), Miller (1987, character 15), De Jong *et al.* (1996, character 66). Antennae are scaled in Parnassiini and Graphiini (Miller, 1987).

All other Papilionini have naked antennae (Ford, 1944).

4- Antennal club: 0 = clavate (gradually thickening towards tip), 1= not clavate (capitate, with a prominent club).

Ford (1944) and Hancock (1983). Most Papilionidae, including *Archon, Luehdorfia, Bhutanitis, Zerynthia*, etc. have elongate antennae that gradually thicken and form a club at the tip. In *Baronia, Parnassius, Hypermnestra,* etc. the club is more prominent (Ford, 1944; Hancock, 1983).

5- Grooves on antennal segments: 0 = without lateral or mesial grooves or depressions, 1 = with more or less wide basal mesial depressions, 2 = paired lateral depressions, 3 = with paired lateral grooves, 4 = with a longitudinal mesial groove or depression.

Miller (1987, character 108); modified by De Jong *et al.* (1996, character 69).

6- Smoothness of antennal segments: 0 = smooth, 1 = serrate.

Saigusa and Lee (1982) pointed out that the antennal segments are serrate in *B.mansfieldi*. In the present study, this character was verified and found to be an autapomorphy of *B. mansfieldi* as no other species had serrate antennae.

7- Raised ventral carinae on antennal flagellum: 0 = absent, 1 = three carinae separating two grooves or paired depressions.

De Jong *et al.* (1996, character 70) and Ackery *et al.* (1999). Present in Nymphalidae only.

8- **Tentorial arms:** 0 = simple, 1 = low-crested, 2 = high-crested, 3 = curved.

Ehrlich (1958) and Miller (1987, character 45).

9- **Eye setae:** 0 = apparently bare, 1 = conspicuously setose.

Ehrlich (1958). The eyes in most Nymphalidae, Lycaenidae, etc. are covered with setae (Higgins, 1975). Members of the Papilionidae possess bare eyes (Ehrlich, 1958); the exception to this is *Bhutanitis mansfieldi*, which has setose eyes (Saigusa and Lee, 1982).

10- **Eye margin:** 0 = entire, not emarginate, 1 = weakly to strongly emarginate adjacent to insertion of antennae.

Ehrlich (1958), De Jong *et al.* (1996, character 73), and Ackery *et al.* (1999). Eyes are emarginate in Lycaenidae.

11- **Eye ring:** 0 = absent, 1 = partial glossy eye ring lacking distinct ommatidial facets, 2 = complete eye ring with reduced ommatidial facets.

De Jong *et al.* (1996, character 74). Re-examination of this character showed that it is present in *Parnassius,* in contrast to the findings of De Jong *et al.* (1996: 83).

12- Lateral scale tuft ("eyelash") extending over eye from antennal base: 0 = absent, 1 = present.

De Jong *et al.* (1996, character 71). Present in Hesperiidae.

13- Labial palpus length: 0 = relatively short (less than twice the length of the head), 1 = relatively long (more than twice the length of head).

Used by several workers (e.g. Ford, 1944; Hancock, 1983; Ehrlich, 1958; Miller, 1987; De Jong *et al.*, 1996 (characters 75 and 76); Ackery *et al.*, 1999). As illustrated by Miller (1987, characters 12 and 113), most Papilionidae, Parnassiinae, and *Baronia* have a short palpus, particularly because of the short terminal segment, and in *Troides* and *Parides* the palpus is two segmented (Miller, 1987; character 113). In Zerynthiinae and *Luehdorfia* the palpi (and the terminal segment) are long, while it is relatively short in *Archon*.

14- Basal palpal segment with a large medial flap: 0 = absent, 1 = present.

Miller (1987, character 35).

b) Adult thorax:

15- Lateral plates of thoracic pronotum: 0 = fused mediodorsally, 1 = not fused.

Ehrlich (1958), De Jong *et al.* (1996, character 86) and Ackery *et al.* (1999).

16- **Serrated ridges on the mesothorax:** 0 = absent, 1 = present. Le Cerf (1913) and others (Häuser, 1993; Korb, 1997) noted this as an autapomorphy for *Hypermnestra*. These are a pair of longitudinally oriented large serrated ridges that appear on the dorsal part of the mesothorax in *Hypermnestra*.

17- **Anepisternum of mesothorax:** 0 = well developed (1/3 to 1/4 length of whole episternum), 1 = small (1/4 or less of episternal length), 2 = present only as a tiny sclerite, 3 = not clearly discernable, or absent.

Ehrlich (1958), De Jong *et al.* (1996, character 87) and Ackery *et al.* (1999).

18- **Precoxal (paracoxal) and marginopleural sutures:** 0 = not fused, 1 = fused.

De Jong *et al.* (1996, character 88).

19- **Precoxal suture:** 0 = reaching anterior margin of basisternum (delimiting triangular basisternum), 1 = not reaching anterior margin of basisternum, 2 = not clearly discernable or absent. Ehrlich (1958) and De Jong *et al.* (1996, character 89).

20- **Parepisternal suture:** 0 = strongly curved before reaching the margin of basisternum or co-linear; 1 = Extending straight or in smoothly curved line from dorsal end to base of spinasternum. De Jong *et al.* (1996, character 90) and Ackery *et al.* (1999).

21- **Parepisternal suture:** 0 = well-developed, 1 = present only as a shallow depression, 2 = indistinct or absent.

De Jong *et al.* (1996, character 91).

22- **Secondary sternopleural suture:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 92) and Ackery *et al.* (1999).

23- **Spinasternum:** 0 = not produced laterally, 1 = produced laterally.

Ehrlich (1958), Miller (1987, character 125), De Jong *et al.* (1996, character 93), and Ackery *et al.* (1999).

24- **Mesophragma:** 0 = with indistinct dorsal plates or flat ridges; 1 = with dorsal processes.

De Jong *et al.* (1996, character 94) and Ackery *et al.* (1999).

25- **Prescutum:** 0 = Oblique or almost vertical, with metanotum not appearing truncated; 1 = approximately vertical or with upper end slightly anterior to lower end, mesoscutum appearing truncated in lateral view.

De Jong *et al.* (1996, character 95).

26- **Mesoscutellum:** 0 = not overhanging metanotum; 1 = overhanging metanotum.

De Jong *et al.* (1996, character 96) and Ackery *et al.* (1999).

27- **Tegula attached to mesonotum by membrane:** 0 = remote from ventral edge, 1 = at ventral edge.

De Jong *et al.* (1996, character 97) and Ackery *et al.* (1999).

28- **Secondary sclerite posterior to metascutellum:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 98) and Ackery *et al.* (1999).

29- **Abdominal sternite 2:** 0 = without distinct anterior sclerite (at most slight sclerotization in membrane), 1 = with narrow anterior sternite separated by weak sulcus.

Ehrlich (1958), De Jong *et al.* (1996, character 100) and Ackery *et al.* (1999).

30- **Patagium:** 0 = well sclerotized, 1 = not well sclerotized.

Ehrlich (1958), Hancock (1983) and Miller (1987, character 22). In *Archon, Hypermnestra, Parnassius,* and *Luehdorfia,* patagia are well developed, while all other Zerynthiini have membranous patagia (Ehrlich, 1958). In Papilioninae, patagia are membranous (*Papilio, Battus*) or nearly so (*Troides, Parides, Baronia*), or well sclerotized (*Graphium*). Scudder (1875) discussed the "paraptera" on *Thaites ruminiana* which is another name for patagia in Lepidoptera (Torre-Bueno, 1985).

31- **Cervical sclerites:** 0 = not joined, 1 = joined ventro-medially. Ehrlich (1958), Miller (1987, character 4) and Ackery *et al.* (1999). "In all Papilionidae, these sclerites are joined beneath the neck by a narrow sclerotic band, a condition that does not occur in other butterflies" (Miller, 1987: 377).

32- **Ventral sclerite on cervical membrane:** 0 = absent, 1 = present. Ehrlich (1958), Miller (1987, character 8) and Ackery *et al.* (1999). According to Miller, *Parnassiinae* and *Papilioninae,* but not *Baronia*, share this feature.

33- **Distinct meral suture on metathorax:** 0 = absent, 1 = present. Miller (1987, character 20) and Ackery *et al.* (1999).

34- **Spine on prodiscrimen:** 0 = absent, 1 = present. Ehrlich (1958) and Miller (1987, character 32).

35- **Lamella of metadiscrimen connecting to furca:** 0 = at the base, 1 = high.

Ehrlich (1958) and Miller (1987, character 33).

c) Legs:

Legs are *Parnassius*-like in *Doritites* (Rebel, 1898), absent in *Praepapilio* fossils; and in *Thaites*, "they are not well enough preserved to state anything concerning them with certainty" (Scudder, 1875: 57).

36- **Male forelegs:** 0 = similar to mid- and hind-legs, 1 = reduced.

Ehrlich (1958), De Jong *et al.* (1996, character 43) and Ackery *et al.* (1999).

37- **Scales on legs:** 0 = absent, 1 = present.

Munroe (1961) and Miller (1987, character 16). The legs in Zerynthiini, Troidini, and *Papilio* are bare; but covered with scales in in *Baronia*, Parnassiini, Graphiini, *Teinopalpus*, etc.

38- **Male foreleg:** 0 = with 5 tarsomeres, 1 = with only 1 tarsomere.

De Jong *et al.* (1996, character 47).

39- **Female foreleg:** 0 = with 5 tarsomeres, 1 = with only 1 tarsomere.

De Jong *et al.* (1996, character 48).

40- **Dorsal spines on foreleg tarsomeres:** 0 = absent, 1 = few, 2 = many.

De Jong *et al.* (1996, character 49). In this context, "few" means sparsely distributed and in low frequency (up to 5 on each tarsomere), and "many" means densely distributed (>10 on each tarsomere). Upon re-examination, *Baronia* *brevicornis* was coded as having "many" spines despite coding as having "few" spines by De Jong *et al.* (1996).

41- **Fore-tibial epiphysis:** 0 = absent, 1 = present, reaching end of tibia, 2 = present, not reaching end of tibia.

Ehrlich (1958), Munroe (1961), Higgins (1975), De Jong *et al.* (1996, character 45), and Ackery *et al.* (1999). Present in all Papilionidae, absent in Pieridae, Lycaenidae, and Nymphalidae. Epiphysis is reduced in size in *P. clodius* and *P. phoebus* (Bryk, 1935; Munroe, 1961).

42- **Fore-tibial spines:** 0 = absent, 1 = few, 2 = many.

Miller 1987 (characters 100 and 131); modified by De Jong *et al.* (1996, character 46). Miller notes the spine-covered swollen hind tibia of males (his character 131) and a row of closely spaced spines running the length of the tibia in females of Troidini (his character 100). In this context, "few" means sparsely distributed and in low frequency (up to 5 on each tibia), and "many" means densely distributed (>10 on each tibia).

43- **Terminal structure of male foreleg:** 0 = asymmetrical claws, 1 = symmetrical claws, 2 = lacking claws, terminal tarsal segment blunt or papillate, 3 = lacking claws, terminal tarsal segment curved downward to a point.

Used by many workers (Ehrlich, 1958; Saigusa and Lee, 1982; Hancock, 1983; Scott, 1984; Häuser, 1993; De Jong *et al.*, 1996 [character 50]; Ackery *et* *al.*, 1999). Asymmetrical tarsal claws are present in males of most Parnassiinae; as well as in the Papilioninae genus *Parides* (Hancock, 1983). The degree of asymmetry varies in *Bhutanitis* (Saigusa and Lee, 1982). Häuser (1993) pointed out that *Hypermnestra* has symmetrical claws, despite statements to the contrary (Scott, 1984; Hancock, 1983).

44- **Terminal structure of female foreleg:** 0 = asymmetrical claws, 1 = symmetrical claws, 2 = lacking claws, terminal tarsal segment bluntly clubbed or ankylose.

De Jong *et al.* (1996, character 51).

45- **Claws on prothoracic legs:** 0 = curved, 1 = almost straight.

De Jong *et al.* (1996, character 52), also Carbonell (1996). Upon re-examination, claws were determined as almost straight in *Parnassius* and curved in *Baronia* despite coding to the contrary by De Jong *et al.* 1996. Carbonell (1996) also discussed the difference in color of prothoracic legs in species of the genus *Allancastria*, but this trait was found to be somewhat variable and therefore not used here.

46- **Claws on prothoracic legs:** 0 = simple, 1 = distinctly bifid.

Ehrlich (1958); Miller (1987, character 39), De Jong *et al.* (1996, character 53), and Ackery *et al.* (1999).

47- **Aroliar pad of claws on pterothoracic legs:** 0 = absent, 1 = small and vestigial, 2 = large and well developed.

Ehrlich (1958) and De Jong *et al.* (1996, character 54). Upon re-examination, a large and conspicuous aroliar pad was observed in *Parnassius*, although De Jong *et al.* 1996 code this character as "missing".

48- **Pulvilli of claws on pterothoracic legs:** 0 = absent, 1 = present.

Ehrlich (1958); Miller (1987, character 2) and De Jong *et al.* (1996, character 55). Higgins (1975) indicated that these structures are absent in Papilionidae and most Pieridae, and are present in Hesperiidae, some Pieridae, Lycaenidae, and Nymphalidae. Miller notes that "the trait requires further study" (1987: 377).

49- **Spines on tibiae of pterothoracic legs:** 0 = absent, 1 = few, 2 = many.

De Jong *et al.* (1996, character 56). In this context, "few" means sparsely distributed and in low frequency (up to 5 on each tibia), and "many" means densely distributed (>10 on each tibia).

50- **Dorsal spines on tarsi of pterothoracic legs:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 57) and Ackery *et al.* (1999).

51- **Hind tibia terminal spurs:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 58). Upon re-examination, terminal spurs on the hind tibia were observed in *Parnassius* despite coding as absent by De Jong *et al.* (1996).

52- **Mid-tibia sub-basal scale brush:** 0= absent, 1 = present.

De Jong *et al.* (1996, character 59).

53- **Mid-tibial hair pencil on hindleg:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 60).

54- **Hindleg mid-tibial spurs:** 0 = absent, 1 = present.

Ehrlich (1958), De Jong *et al.* (1996, character 61) and Ackery *et al.* (1999). Ackery *et al.* (1999: 274) pointed out that the absence of this character, "proposed by Minet (1991) and others, is certainly not unique [to Papilionoidea]; a comparable condition occurs in [some] Hesperiidae".

d) Basal structures of wings of the adult:

Not preserved (or at least not discussed by the authors) in all three fossil butterflies.

55- **Trypanum:** 0 = absent, 1 = vestigial, 2 = present.

Le Cerf (1913), noted by Dujardin (1967) (who first used the term "trypanum" for the structure) and Korb (1997). This is a forked, chitinized structure at the base of the forewing which is unique to *Hypermnestra*, but is present in a reduced form in some *Parnassius* species.

56- **Axilliary 3:** 0 = reaching forward not further than halfway to base of vein 1A+2A, 1 = reaching forward almost to or beyond anterior corner of 1A+2A. De Jong *et al.* (1996, character 78), and Ackery *et al.* (1999).

57- **Axilliary 3:** 0 = irregularly Y-shaped, 1 = differently shaped. De Jong *et al.* (1996, character 79), and Ackery *et al.* (1999). Only in Hesperiidae, axilliary 3 is irregularly Y-shaped (Ackery *et al.*, 1999).

58- **Outer margin of axilliary 3:** 0 = more or less straight or convex, 1 = strongly concave. De Jong *et al.* (1996, character 80).

59- **Outer margin of axilliary 3:** 0 = smooth, without any indentation or extension, 1 = with blunt tooth or indentation, on which base of vein 1A+2A hinges.

De Jong *et al.* (1996, character 81) and Ackery *et al.* (1999).

60- **Posterior part of axilliary 3:** 0 = connected to base of vein 2A; 1 = not connected to base of vein 2A.

De Jong *et al.* (1996, character 82).

61- **Median plate 1:** 0 = free, 1 = fused with axilliary 3.

De Jong *et al.* (1996, character 83).

62- **When viewed directly from above, median plate 2:** 0 = partly covered by base of vein 1A+2A, 1 = invisible or nearly so.

De Jong *et al.* (1996, character 84).

63- **When viewed directly from above, radial plate:** 0 = not covering part of axilliary 2 and median plate 1; 1 = raised and partly covering axilliary 2 and median plate 1.

De Jong *et al.* (1996, character 85).

e) Adult wings:

64- **General appearance of typical scales on the wings:** 0 = normal, 1 = elongate, 2 = round, 3 = square, 4 = teardrop shaped.

Le Cerf (1913), Hiura (1980) and Miller (1987).

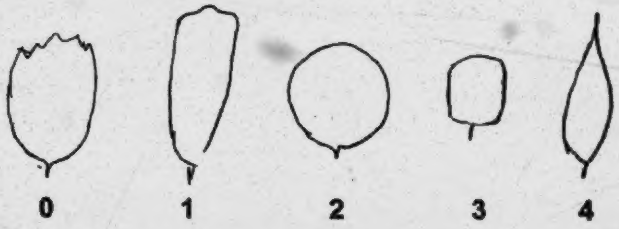

Fig. 1. Typical scales on the surface of the wings, as coded above.

65- **Pattern between longitudinal ridges of wing scales:** 0 = ladder-like, 1 = reticulate.

Miller (1987, character 24).

66- **Male androconial scales:** 0 = absent, 1 = present. Miller (1987; characters 91, 105, 157, 166).

Miller (1987) argues that homologous male-specific scent scales, otherwise known as androconia, are present in all papilioninae despite previous suggestions to the contrary (Miller under character 18). He presents a detailed examination of types of these scales and their locations on the wings, summarized here under the next character.

67- **"Anal brushes" [=bristles, =hair-like scales] along vein 2a on ventral surface of male hindwing:** 0 = absent, 1 = present.

Characters by Miller (1987, character 18). Present in all Papilioninae.

68- **Pocketed brush in anal cells of hindwing (anal fold) of males:** 0 = absent, 1 = present.

Miller (1987, character 101) and De Jong *et al.* (1996, character 21).

Wing Venation:

f) Forewing venation:

69- **Forewing discal cell:** 0 = intermediate in size, 41-59% of the wing length, 1 = long, 60% or more of the wing length, 2 = short, 40% or less of the wing length.

Hancock (1983) and Miller (1987, character 26), modified by De Jong *et al.* (1996, characters 10 and 11). The character states used here are as they appear in De Jong *et al.* 1996. Hancock and Miller use simpler terms; Miller used "Forewing discal cell less than half the length of the forewing" for Teinopalpini.

70- **Forewing discal cell:** 0 = fully closed, 1 = weakly closed.

Ehrlich (1958) and De Jong *et al.* (1996, character 14). In Hesperiidae and Lycaenidae, the vein closing the discal cell on forewing is much weaker than other veins on fore wing.

71- **Forewing middle discocellular vein (mdc):** 0 = straight, 1 = incurved.

Miller (1987, character 13). This vein is curved inwards in Parnassiinae, *Teinopalpus and Meandrusa*.

72- **Forewing discocellular recurrent veins:** 0 = absent, 1 = weakly present, 2 = distinctly present.

De Jong *et al.* (1996, character 12).

73- **Forewing 2A:** 0 = present, converged with 1A and not reaching margin, 1 = present, free and reaches wing margin, 2 = absent.

Miller (1987, character 3), Heppner (1998) and De Jong *et al.* (1996, character 16). Separates Papilionidae (state 1) from all other butterflies (state 0). 2A is absent in Satyrinae (De Jong *et al.*, 1996).

74- **Forewing radial venation:** 0 = reduced (4 veins), 1 = normal (5 veins).

Ford (1944), Ehrlich (1958), Hiura (1980, character 6), Miller (1987, character 6), and Ackery *et al.* (1999).

75- **Number of forewing radial veins arising from discal cell:** 0 = five, 1 = four, 2 = three, 3 = two.

De Jong *et al.* (1996, character 4).

76- **Forewing origin of R3:** 0 = stalked with R4+5, 1 = arising from base of R4+R5, 2 = stalked or anastomosing with R2.

Munroe (1961), perhaps based on earlier work (e.g. Talbot 1939); also Ackery (1975), Hancock (1983), and Miller (1987, character 27 and 75). R3 arises from the base of R4+5 in *Sericinus* and *Hypermnestra*; it is variable in *Parnassius*; and is stalked with R2 in *Thaites*. In *Lamproptera* R3 and R4 are long-stalked, and R5 arises from R3+4 just beyond cell (Miller, 1987).

77- **Forewing R1 and Sc:** 0 = fused, 1 = separate.

Miller (1987, character 6) based on work by Ford (1944) and others. In *Baronia*, *Graphium* and some *Eurytides*, R1 and Sc are fused.

78- **Pattern of forewing cubital system:** 0 = quadrifid, 1 = trifid.

De Jong *et al.* (1996, character 8) and Ackery *et al.* (1999).

79- **Forewing median (basal) spur from Cu:** 0 = absent or rudimentary; 1 = present.

Ford (1944), Munroe (1961), Hiura (1980, character 9), and Miller (1987, character 19). This spur is absent in *Baronia*, *Zerynthia, Luehdorfia, Parnassius, Hypermnestra, Archon* and is present in all Papilionidae except *Teinopalpus*. As noted both by Ford (1944) and Munroe (1961), the spur is vestigial in *Bhutanitis* and *Sericinus*.

80- **Forewing origin of M1:** 0 = stalked with R2-5, 1 = upper angle of cell, 2 = below upper angle of cell.

Munroe (1961). Miller (1987, character 71) used this character in the following form: "Forewing upper discocellular (udc) longer than middle discocellular (mdc)".

81- **Forewing veins M1 and M2:** 0 = not incurved towards wing base, 1 = slightly incurved, 2 = incurved.

De Jong *et al.* (1996, character 13). Character rescored here.

82- **Forewing veins M2 and M3:** 0 = not incurved, 1 = slightly incurved, 2 = incurved, 3 = sharply angled.

De Jong *et al.* (1996, character 15). Character rescored here.

g) Hindwing venation:

83- Hindwing discal cell: 0 = intermediate, 41-59% of wing length, 1 = long, 60% or more of wing length, 2 = short, 40% or less of win length. Munroe (1961) and Miller (1987, character 73); modified by De Jong *et al.*, (1996, characters 32 and 33). The character states used here are as they appear in De Jong *et al.* (1996). Munroe and Miller use simpler terms; Miller used "Hind wing discal cell small" for *Lamproptera*.

84- Hindwing discal cell: 0 = strongly closed, 1 = weakly closed. Ehrlich (1958) and De Jong *et al.* (1996, character 34).

85- Hindwing discocellular recurrent veins: 0 = absent, 1 = present. De Jong *et al.* (1996, character 35).

86- Hindwing humeral (=precostal) vein: 0 = absent, 1 = present. Higgins (1975) and Scott (1984). This vein is absent in Lycaenidae, Hesperiidae and some Pieridae, and present in all other butterflies.

87- Hindwing humeral (=precostal) vein: 0 = branched (forked), 1 = unbranched (simple).

Ford (1944) and Munroe (1961) indicate that this vein is branched in *Baronia, Luehdorfia, Sericinus*, etc., and unbranched in *Bhutanitis, Zerynthia, Parnassius, Hypermnestra,* and *Archon*. However, no branching was observed in *Sericinus*, and the vein was found to be branched in most *Parnassius* species. In *Euryades* the vein is club-shaped (Miller, 1987).

88- **Hindwing humeral (=precostal) vein bent:** 0 = distad, 1 = basad.

Munroe (1961).

89- **Hindwing humeral (=precostal; =basal) cell:** 0 = narrow or rudimentary, 1 = wide.

Ford (1944), Munroe (1961), Hiura (1980, character 18), Miller (1987, character 127), and De Jong *et al.* (1996, characters 30 and 31). The cell is narrow in higher Papilionidae, *Zerynthia, Luehdorfia, Parnassius, Hypermnestra, Archon*, etc., and wide in *Baronia, Sericinus, Bhutanitis*, etc.

90- **Hindwing origin of Rs:** 0 = Distinct from Sc, 1 = fused with SC at base.

Ackery *et al.* (1999).

91- **Hindwing area of small specialized scales adjacent to humeral cell in hindwing:** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 26) and Ackery *et al.* (1999).

92- Hindwing vein 1A: 0 = present, 1 = absent.

Ford (1944), Munroe (1961), Miller (1987, character 7), Heppner (1998), De Jong *et al.* (1996, character 20), and Ackery *et al.* (1999). The 1st A in hindwing (3rd A according to some authors) is absent in *Parnassiinae* and *Papilioninae*, and is present as a tubular vein in non-Papilionidae, *Baronia*, and *Praepapilio.*

93- Hindwing prominent groove: 0 = absent, 1 = present.

Miller (1987, character 104).

94- Hindwing vein 1A+2A: 0 = not swollen, 1 = swollen.

Miller (1987, character 106).

95- Hindwing vein 1A+2A: 0 = larger than Cu2A, 1 = nearly same size as Cu2A, 2 = shorter than Cu2A.

Miller (1987, character 117).

96- Hindwing cross-vein M1-M2 (mdc): 0 = forming angle with M2, 1 = curving into M2 smoothly, 2 = running more or less straight into M2.

De Jong *et al.* (1996, character 36).

97- **Hindwing M2:** 0 = absent, 1 = present, or weakly present. Higgins (1975) and De Jong *et al.* (1996, character 24). The vein is absent in subfamily Hesperiinae (*Hylephila* in this study), and present in all other butterflies. M2 on hindwing is weakly present only in *Pyrgus* (De Jong *et al.*, 1996).

98- **Hindwing origin of M2 and M3:** 0 = approaching, 1 = distant. Hiura (1980, character 17). The lower discocellular vein can be either smaller than (0) or same size of (1) middle discocellular vein, reflecting on the distance between the bases of veins M2 and M3 on Hindwing.

99- **Hindwing M3 and Cu1A:** 0 = well separated, 1 = united. De Jong *et al.* (1996, character 28). De Jong (1996) uses the term "connate" for state 1.

100- **Hindwing space Rs:** 0 = with parallel sides, 1 = narrows toward outer margin. Hiura (1980, character 20).

101- **Hindwing M2 elongate and forms a well-marked tooth:** 0 = absent, 1 = present.

Ford (1944).

102- Hindwing M3 elongate and forms a tail (or at least a well marked tooth): 0 = absent, 1 = present.

Ford (1944), and used by many other workers following him. Ford differentiated the types of the tails produced by M3 by using the terms “definite” (as in *Luehdorfia*) and “dentations” (as in *Zerynthia*), but here they are considered together. The elongate hindwing tail in some Lycaenidae and Hesperiidae are not homologous with those in Papilionidae, as they are the result of elongation of CuA2 and the anal vein respectively.

103- Tip of tail on hindwing M3: 0 = same with or narrower than base and fine, 1 = wider than base or clublike. New character. The tail is clublike in *B.mansfieldi* only.

104- Hindwing cross-vein between Rs and M1 (udc): 0 = straight, 1 = sinuate.

Miller (1987, character 46).

105- Hindwing Cu1A elongate and forms a tail: 0 = absent, 1 = present.

De Jong *et al.* (1996, character 39).

106- Hindwing Cu2A elongate and forms a tail: 0 = absent, 1 = present.

De Jong *et al.* (1996, character 38).

107- Hindwing tornal lobe (margin of cell Cu2A): 0 = absent, 1 = weak, 2 = strong.

Ford (1944), Hiura (1980, character 11), Miller (1987, character 136), and De Jong *et al.* (1996, character 41). A strong indentation is present in *Luehdorfia* and *Bhutanitis mansfieldi*, as well as males of subgenus *Losaria* of *Pachliopta* (Miller, 1987). *Parnassius, Hypermnestra,* and *Archon* have entire margins (Ford, 1944).

108- Hindwing anal cell: 0 = normal, 1 = enlarged.

Miller (1987, character 144). In Troidini, the anal cell is exceptionally large (state 1), sometimes forming a "pocket" (Tyler *et al.,* 1994). Other butterflies do not show such an enlargement (state 0).

h) Wing pattern: Characters in this section (109-160) are either new for this study, or modified from Hiura (1980) as indicated. Hiura presents generalized wing pattern schemes and an easy method to score the character on the wings of Parnassiinae (1980) and Papilionini (1981), but he does not homologize these characters with eachother. Some corrections to Hiura's coding are made here. To facilitate uniform coding of wing pattern elements that are compatible with previous studies, a generalized wing pattern for Papilionidae was constructed (Fig 2), although the proposed model was not extended to include outgroups due to difficulties in unambiguously assigning character states. Therefore, the character states are coded as “not present” in the character matrix for the six outgroup taxa.

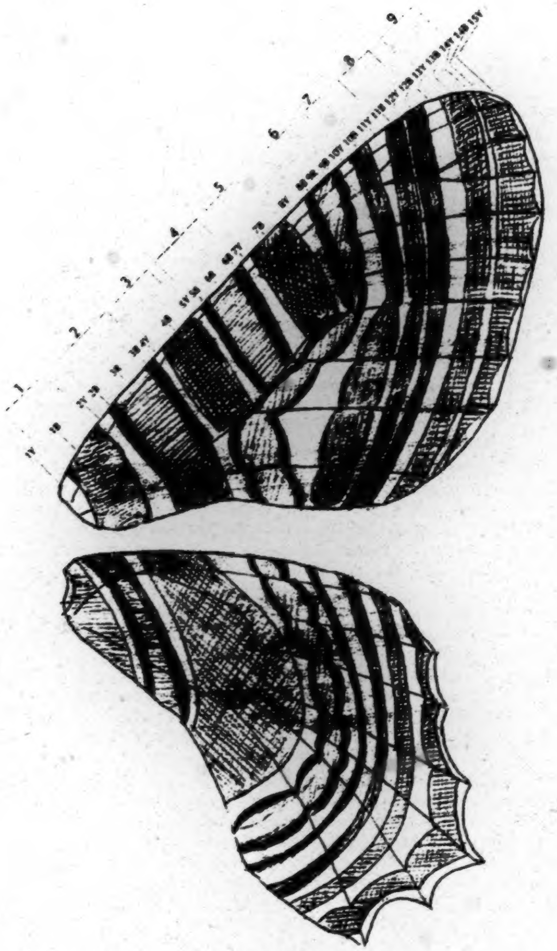

Fig 2. Wing pattern proposed here.

Upperside of Forewing:

109- **Forewing pattern:** 0 = visible, 1 = concealed, the wing being uniformly dark.

110- **Band 2 in cell:** 0 = absent (or mostly absent), 1 = present.

In *Sericinus* and *Parnassius*, band 2 is usually absent in males.

111- **Red mark inside band 2:** 0 = absent, 1 = present.

Hiura's (1980) character 26. Present in *Zerynthia rumina* only.

112- **Band 3 in cell:** 0 = absent, 1 = present.

113- **Band 4 in cell:** 0 = absent (or mostly absent), 1 = present (or mostly present).

In *Sericinus*, band 4 is only present in females.

114- **Red mark inside band 4:** 0 = absent, 1 = present. Hiura's character 28.

Present in *Zerynthia rumina* only.

115- **Band 6:** 0 = nearly straight, 1 = bent or cut in vein M3, lower half often merged with band 3.

116- **Red mark inside upper portion of band 6:** 0 = absent (or mostly absent), 1 = always present.

Hiura's (1980) character 31. Present in *Zerynthia rumina, Archon, Sericinus,* *Hypermnestra,* and *Parnassius*.

117- Distinct black dot of band 6 in space Cu2A: 0 = absent, 1 = present.

Present in females only in *Hypermnestra* and *Allancastria*.

118- Red mark inside the black dot of band 6 in space Cu2A: 0 = absent, 1 = present.

Present in *Z. rumina,* a number of *Parnassius* species, and occasionally in *Sericinus*.

119- Upper half of band 6 and lower half of band 7: 0 = separate, 1 = fused.

120- Distinct round dots of Band 7 in spaces M3 and Cu2A: 0 = absent, 1 = present.

121- Band 8: 0 = entire, 1 = divided. Hiura's (1980) character 34.

122- Band 9: 0 = simple (entire), 1 = modified.

123- Bands 5 and 7 in space M3: 0 = separate, 1 = fused.

Upperside of Hindwing:

124- Band 3 in cell: 0 = absent, 1 = present.

Hiura's (1980) character 50. Not to be confused with band 1, which extends along inner margin.

125- Band 1 along inner margin: 0 = narrow, does not enter cell; 1 = wide, enters cell. Hiura's (1980) characters 47 and 49.

126- Band 2 basal red mark inside the cell on Hindwing: 0 = absent, 1= present.

Present in *Zerynthia rumina* (upper-and underside) and some *Parnassius*.

127- Position of band 4 element in space M1: 0 = postdiscal (basad), 1 = middle of cell or submarginal (distad).

Hiura's (1980) character 52. Band 4 (often with marked red elements) is normally positioned submarginally (*Allancastria, Zerynthia, Bhutanitis, Sericinus, Luehdorfia*). It is closer to the cell in *Parnassius* and to the margin in *Archon*.

128- Band 4 red spot in cell R5: 0 = absent, 1 = present.

Hiura's (1980) character 51. Present in *Archon, Hypermnestra, Parnassius,* and *Sericinus*. Absent in all other Zerynthiinae.

129- **Band 6 along outer margin:** 0 = entire, 1 = divided. Hiura's (1980) character 55.

130- **Blue submarginal spots:** 0 = absent, 1 = present, without white center, 2 = present, with white center.

Munroe (1961) for subgenus *Parnassius*; and Hiura (1980, character 23). Carbonell (1997) mentioned the absence of these scales in *Allancastria deyrollei* and *A.louristana*, and their presence in other *Allancastria*. Hiura (1980) only excludes *Hypermnestra* from having these scales.

Underside of Forewing:

131- **Band 2 in cell:** 0 = absent (or mostly absent), 1 = present. Hiura's character 38.

132- **Red mark inside band 2:** 0 = absent, 1 = present.

133- **Band 4 in cell:** 0 = absent (or mostly absent), 1 = present. Hiura's (1980) character 40.

134- **Red mark inside band 4:** 0 = absent, 1 = present.

135- **Band 5 in Cu2A and 1A (under band 2):** 0 = absent, 1 = present. Hiura's (1980) character 41.

136- **Red mark inside upper portion of band 6:** 0 = absent, 1 = present.

137- **Red mark inside spot of band 6 in space Cu2A:** 0 = absent, 1 = present.

138- **Bands 7 and 8:** 0 = separate, 1 = fused. Hiura's (1980) character 43.

139- **Band 8:** 0 = entire, 1 = divided.

140- **Band 9:** 0 = simple (entire), 1 = divided into two distinct bands, 2 = modified.

Underside of Hindwing:

141- **Band 1 in space 2A:** 0 = separate from band 3, 1 = fused with band 3 along inner margin. Hiura's (1980) character 57.

142- **Band 2 red element in base of coastal cell:** 0 = absent, 1 = present.

143- **Band 2 red element in base of R1:** 0 = absent, 1 = present.

144- **Band 2 red element in base of discal cell:** 0 = absent, 1 = present.

145- **Band 2 red element in base of Cu2A:** 0 = absent, 1 = present.

146- **Band 3 in cell:** 0 = entire, 1 = divided into smaller pieces. Hiura's (1980) character 60.

147- **Band 4 red element in coastal cell:** 0 = absent, 1 = present.

148- **Band 4 red element in cell R1:** 0 = absent, 1 = present.

149- **Band 4 red element in cell R5:** 0 = absent, 1 = present.

150- **Band 4 red element in cell M1:** 0 = absent, 1 = present.

151- **Band 4 red element in cell M2:** 0 = absent, 1 = present.

152- **Band 4 red element in cell M3:** 0 = absent, 1 = present.

153- **Band 4 red element in cell Cu1A:** 0 = absent, 1 = present.

154- **Band 4 red element in cell Cu2A:** 0 = absent, 1 = present.

155- **Bands 4 and 5:** 0 = separate, 1 = fused. Hiura's (1980) character 61.

156- **Bands 5 and 6:** 0 = separate, 1 = fused, or band 5 indistinct.

157- **Blue submarginal spots of band 5:** 0 = absent, 1 = present.

158- **Band 6:** 0 = entire, 1 = divided into two distinct bands.

159- **Filling of band 6:** 0 = yellow, 1 = orange, 2 = yellow, replaced with blue towards anal margin; 3 = red. Hiura's (1980) character 68.

160- **Marginal crescents:** 0 = absent or inconspicuous, 1 = present.

i) Male genitalia:

There are some inconsistencies with regard to nomenclature of genitalia structures. Character nomenclature mostly follows Klots (1970), but it is contrasted, whenever possible, with the nomenclature of Kuznetsov (1967), Higgins (1975), Miller (1987), Smith and Vane-Wright (2001), and other workers. The genitalia of fossil butterflies could not be discussed, except the presence of a well marked sphragis in *Praepapilio* and *Doritites*.

Tegumen and Uncus:

161- Pseuduncus (=superuncus; = uncus anticus) on 8th tergite: 0 = absent, 1 = present, not pointed, 2 = present, pointed, 3 = 8th tergite circularly expanded.

Munroe (1961), Hancock (1983), Miller (1987, characters 17, 67, 111, 114, 169), Stekolnikov and Kuznetsov (2003, character 13), and Ackery *et al.* (1999). Otherwise known as "superuncus" (Kuznetsov, 1967; Higgins, 1975) or "uncus anticus" (Bascombe *et al.*, 1999). Munroe (1961) notes that in *Parnassius apollo* species group, the anterior and lateral margins of the 8th tergite "form a single arc".

162- Tegumen+uncus (=dorsum; = 9th and 10th tergites): 0 = reduced and immovably articulated with 8th tergite (Papilioninae); 1 = fused and well sclerotized, but free from 8th tergite (Parnassiinae, Baroniinae).

Munroe (1961), Hancock (1983), Miller (1987, characters 31 and 138), and Stekolnikov and Kuznetsov (2003, character 14).

163- Tegumen: 0 = almost flattened, 1 = slightly convex, 3 = strongly convex; 3 = with a "bump" but otherwise straight, 4 = membranous or vestigial.

Saigusa and Lee (1982). Tegumen is strongly convex in *B.mansfieldi*, slightly convex in *B.lidderdali*, and almost flattened in *B.thaidiana*.

164- Dorso-median ridges on posterior part of the tegumen: 0 = absent, 1 = present.

Saigusa and Lee (1982). Present in *mansfieldi* and *lidderdali*, and absent in *thaidiana*.

165- Minute spines on the lateral arm of male dorsum: 0 = absent, 1 = present.

Kato (1998).

166- Uncus: 0 = a single projection, or a set of fused projections (=unpaired), 1 = bifid (=paired) or at least divided at apex, 2 = trifid.

Miller (1987, characters 9, 28, 55) and Stekolnikov and Kuznetsov (2003, character 1). Papilioninae and Parnassiinae have bifid uncus. Trifid uncus is present in males of *Eurytides* (Miller, 1987).

As noted by Miller (1987, under character 9), there seems to be some confusion regarding the nomenclature of uncus and the associated pair of socii. Bifid membranous unci in Papilioninae have often been termed socii, or simply tegumen, with the assumption that uncus is vestigial, where pseuduncus has often been termed "superuncus" (Munroe, 1961; Hancock, 1983; Smith and Vane-Wright, 2001) (Zakharov *et al.*, 2004a reference to uncus is correct). Similarly, trifid unci have often been regarded as a single uncus with paired socii (Munroe, 1961). Miller (under character 28) emphasizes that socii are absent throughout Papilionidae. However, this does not seem to be an accurate conclusion, as socii are clearly present in some *Parnassius* species (cf. Munroe's "paired dorsal processes"). See under "socii" (character 168 below).

167- **Type of uncus:** 0 = well developed, in the form of well-sclerotized strong projection(s); 1 = weakly developed, distally membranous and attached to tegumen, sitting on the dorsal part of the anal tube.

Saigusa and Lee (1982). Uncus is small and weakly developed in *B.lidderdali* and *B.thaidiana*, and extremely large and strong in *Luehdorfia*.

168- **Socii (=subuncus, =falx, =brachia):** 0 = absent, 1 = present.

Munroe (1961). Kuznetsov (1967) used the term "subuncus" for socii in Satyrinae, while Higgins (1975) used the term "brachia" for the same, and "falx" for similar structures in Lycaenidae and *Haemaris*. According to Miller (1987, under character 28), socii are absent in all Papilionidae. However, these are present in a number of *Parnassius* species, including *Parnassius clodius*.

169- **Length of uncus relative to vinaculum:** 0 = short, 1 = nearly the same lenght, 2 = long.

Saigusa and Lee (1982) and Miller (1987, character 112). Uncus is relatively shorter than vinaculum in *Sericinus* and longer than vinaculum in *Bhutanitis* and

Luehdorfia.

170- Lobes of uncus: 0 = straight, 1 = bent ventrally.

Miller (1987, characters 41 and 154). Lobes of uncus are bent ventrally (downwards) only in *Eurytides* and *Troides*.

171- Tip of uncus: 0 = blunt or clavate, 1 = pointed, slightly declivous, 2 = pointed, strongly declivous or claw-like, 3 =curved upwards.

Saigusa and Lee (1982). Uncus is blunt or clavate in *Bhutanitis*, and pointed in *Luehdorfia*.

172- Shape of uncal processes: 0 = very thin throughout, 1 = broad at base, 2 = moderate, 3 = very thick throughout, 4 = broad towards tip, 5 = oval.

Saigusa and Lee (1982). Uncus is thin in *Bhutanitis* and thick in *Luehdorfia*.

173- Spatulate process of tegumen between the two lobes of uncus: 0 = absent, 1 = present.

Munroe (1961); illustrated by Higgins (1975). Unique to *Parnassius apollo* species group.

174- Epicosta: 0 = absent, 1 = small, 2 = large.

Saigusa and Lee (1982). This structure has also been termed "appendix angularis" (Bascombe *et al.*, 1999), "dorsolateral sclerite" (Miller, 1987), and "articulatory process of tegument" (Stekolnikov and Korshunov, 2003). It is small in *Bhutanitis* and large in *Luehdorfia*.

175- **Epicosta:** 0 = distinctly separate from dorsum, 1 = fused with dorsum.

Saigusa and Lee (1982). Epicosta is separate from dorsum in *Bhutanitis* and fused with it in *Luehdorfia*.

176- **Epicosta:** 0 = nearly triangular, 1 = clearly Y shaped.

Kato (1998).

Valve:

177- **Type of valve:** 0 = large, broad, complete; 1 = small, narrow, simple.

Ford (1944), Ehrlich (1958), Hancock (1983), and Miller (1987, character 115). Miller used this character as a synapomorphy for *Pachliopta, Euryades, Losaria* and *Cressida*, none of which are represented in this study; but instead, reduction of valve is observed in *Archon* and a few other genera.

178- **Lateral lobe of valve:** 0 = absent, 1 = present.

Miller (1987, character 5) for *Baronia*. Miller (1987) also used other valval autapomorphies that are not incorporated in the present study, e.g. dorso-basal processes in *Cressida, Euryades, Losaria* and *Pachliopta* of Troidini (Miller's character 119) and two distal spines in *Atrophaneura* (Miller's character 162).

179- Tip of valve: 0 = rounded, 1 = narrowed, with a blunt tip, 2 = narrowed, with a pointed tip.

Munroe (1961) and Saigusa and Lee (1982). The tip of the valve is narrowed with a blunt tip in *apollo* group of *Parnassius* (Munroe's subgenus *Parnassius*); narrowed with a pointed tip in *B.mansfieldi* and a blunt tip in *B.lidderdali.* In *B.thaidiana,* valva ends in a truncate narrow lamella with a short spine-like projection arising from subapical portion of the outer wall of valva.

180- Dorsal margin of valve: 0 = flat, not raised, and often reduced in size; 1 = strongly raised.

Kato (1998) for *Luehdorfia,* but is also applicable to other genera. Valve is reduced in size as a whole in *L.chinensis*, and strongly raised in other *Luehdorfia* species.

181- Dorsal terminal process of valve: 0 = absent, 1 = present.

182- Ventral terminal process of valve: 0 = absent, 1 = present.

Miller (character 149).

183- Harpe: 0 = absent, 1 = simple, 2 = complex.

Miller (1987, characters 60, 81, 116, 132, 155, 156). Absent in *Luehdorfia* *japonica*, despite statement by Saigusa (1973).

184- Stout spines on ventro-basal portion of inner surface of male valve: 0 = absent, 1 = short, 2 = elongate.

Kato (1998), perhaps corresponding to Miller's (1987) character 93 for *Graphium* ("Thickened setae [present] on inner rim of valve").

185- Valve dorso-lateral sclerite: 0 = absent, 1 = small, lightly scleotized, and free-floating, 2 = large, heavily sclerotized, and attached to the valve at the dorsal junction of the valve and tegument. Miller (1987, characters 48 and 56).

186- Valve sclerotized ridges (=crista): 0 = absent, 1 = reduced, 2 = present. Stekolnikov and Kuznetsov (2003, character 4).

187- Shape of juxta (=furca): 0 = primarily V-shaped, 1 = primarily triangular or saddle-shaped, 2 = primarily oval and elongate, 3 = primarily T-shaped.

Character coded mostly using Miller's (1987) illustrations. Structure is termed "furca" by Higgins (1985).

188- Apex of male juxta: 0 = not pointed, 1 = weakly pointed, 2 = strongly pointed. Miller (1987, character 122) and Kato (1998). Character coded mostly using Miller's (1987) illustrations.

189- **Setae on juxta:** 0 = absent, 1 = present.

Miller 1987 (characters 80, 158), and coded mostly using his illustrations.

Aedeagus (=phallus; Kristensen, 2002):

The word "Aedeagus" used here (as it appears in all literature examined) refers to the structure that should be termed "phallus" according to Kristensen (2002), who states that the true aedeagus is present only in Lepidoptera family Agathiphagidae.

190- **Aedeagus:** 0 = thin and heavily sclerotized (Parnassiinae+some Triodini), 1 = thick and weakly sclerotized.

Miller (1987, character 141), Ackery *et al.* (1999) and Stekolnikov and Kuznetsov (2003, character 8). Saigusa and Lee (1982) also mentioned that aedeagus is thick in *B.thaidiana* and thin in *B.mansfieldi.*

191- **Length of aedeagus:** 0 = short (*Luehdorfia*), 1 = long (*Bhutantis*).

New character, based on occasional indications by some workers (Bryk, 1934, 1935; Hancock, 1983; Carbonell, 1997) as a note-worthy character. The length of aedeagus was compared to the height of tegumen+vinculum in coding this character.

192- **Shape of aedeagus:** 0 = straight, 1 = curved at tip, 2 = slightly curved throughout, 3 = strongly curved throughout.

Miller (1987, characters 43 and 68).

193- **Base of aedeagus:** 0 = normal, not flared; 1 = flared, bell-shaped or funnel-shaped, 2 = narrow and club-like.

Miller (1987, character 47).

194- **Tip of aedeagus:** 0 = apically obtuse, 1 = pointed (*thaidiana, mansfieldi*), 2 = slightly dilated subapically (*lidderdlai*).

Saigusa and Lee (1982), and Miller (1987, character 63). Miller also notes the dorso-ventral depression of aedeagus in *Atrophaneura* (character 163).

195- **Distal opening of aedeagus:** 0 = dorsal, 1 = ventral, 2 = lateral.

Miller (1987, character 83).

Musculature:

Characters dealing with genitalia musculature could not be verified; the states are as they appear in Stekolnikov and Kuznetsov 2003.

196- Articulatory process of tegumen: 0 = primarily absent, 1 = reduced, 2 = well developed.

Stekolnikov and Kuznetsov (2003, character 3).

197- m3: 0 = occupying juxta-vinculal position, 1 = occupying juxta-valval position, 2 = extending from juxta to saccus apex.

Stekolnikov and Kuznetsov (2003, character 9, 10).

198- m4: 0 = attached to bases of costal margins of valve, 1 = attached to transtillia. Stekolnikov and Kuznetsov (2003, character 2).

199- m4: 0 = attached to articulatory processes of tegument [=epicosta], 1 = not attached to articulatory processes of tegumen.

Stekolnikov and Kuznetsov (2003, character 5).

200- Intercalary sclerites: 0 = not detached from articulatory processes, 1 = detached from articulatory processes with m4 inserted into them.

Stekolnikov and Kuznetsov (2003, character 7).

201- Intercalary sclerite: 0 = without apodeme, 1 = with apodeme serving for attachment of m4.

Stekolnikov and Kuznetsov (2003, character 11).

202- Intercalary sclerite: 0 = not fused with sub-anal plate, 1 = fused with sub-anal plate. Stekolnikov and Kuznetsov (2003, character 12).

j) Female genitalia:

203- Female abdomen with von Siebold organ: 0 = absent, 1 = present. Ackery *et al.* (1999). Present in Nymphalidae.

204- Female 8th tergum: 0 = distinct from 8th sternum, 1 = fused with 8th sternum.

Saigusa and Lee (1982). The 8th tergum is distinct from sternum in *Luehdorfia* and fused with it in *Bhutanitis*.

205- Female 8th tergum: 0 = small (*Luehdorfia*), 1 = large (*Bhutanitis*). Saigusa and Lee (1982). The 8th tergum is small in *Luehdorfia* and large in *Bhutanitis*.

206- Female 8th sternum: 0 = small, 1 = large.

Kato (1998).

207- Female ostium bursae: 0 = inconspicuous, with little or no sclerotizations, 1 = with sclerotized invagination, or entirely sclerotized. Munroe (1961) and Miller (1987, characters 23, 44, 84, 88, 134, 152, 153, 159, 160, 167, 169). Ostium bursae is inconspicuous in *Hypermnestra*, and has sclerotized invagination (or is entirely sclerotized) in *Zerynthia, Archon, Sericinus, Allancastria, Graphium, Cressida, Euryades,* etc. Miller used different shapes of this sclerotization as characters, and coined terms for various configurations.

208- **Sides of the ostium bursae:** 0 = almost flattened, 1 = slightly raised, 2 = very much raised.

Saigusa and Lee (1982). Sides of the ostium bursae are almost flattened in *B.lidderdali*, slightly raised in *B. mansfieldi*, and very much raised in *B.thaidiana*.

209- **Upper half of divided female 8th tergum:** 0 = not narrowed, 1 = strongly narrowed. Kato (1998).

210- **Hook-like setae on medial surface of papillae anales:** 0 = absent, 1 = few,

2 = many. Miller (1987, characters 52, 78, 102, 128).

211- **External sphragis (=pouch, = spermatophragma):** 0 = absent, 1 = present.

Ford (1944), and used by many workers. Present in many Parnassiinae, including *Bhutanitis, Parnassius, Luehdorfia,* as well as many Troidini (Miller, 1987), and absent in *Hypermnestra* and *Archon* despite statements to the contrary (see Häuser, 1993; Tyler *et al.*, 1994). It is also absent in *Baronia, Sericinus* and

Zerynthia, but is present in the fossils *Prepapilio* and *Doritites*. A prominent and "alate" sphragis can be seen in *Cressida* and *Euryades* (Miller, 1987; character 121), and a vestigial one in *Atrophaneura proneus* (Ford (1944). Not be confused with the "mating plugs" (="the internal sphragis"), which have a similar function (preventing female from remating), and are present in many papilionids, including *Graphium* and *Papilio* (Matsumoto and Suzuki, 1995; Orr, 1995).

212- **Apophysis anterioris:** 0 = absent, 1 = present, short, 2 = present, long.

Miller (1987, character 77) and Kato (1998). The length of the apophysis anterioris is coded reltive to the overall size of genitalia armature.

213- **Signum:** 0 = completely absent, 1 = present only in the form of concentric folds on corpus bursa, 2 = present, inconspicuous, as a small group of spiracles, 3 = present as a hard plate of normal size; 4 = present, long, thin and zipper-like, 5 = present, long, narrowed, horn-like.

Miller (1987, characters 38, 90, 120, 133, 150).

214- **Signum orientation:** 0 = longitudinally oriented, 1 = dorso-ventrally oriented. Miller (1987, characters 38 and 165).

Although the early stages of *B. ludlowi* are still unknown, some of the life history characters for this species are coded here as it is highly unlikely that these would be dramatically different from other species of Zerynthiini.

k) Egg:

215- **Type:** 0 = spherical, slightly flat at the bottom, 1 = low-dome shaped, 2 = flat, 3=cylindrical.

Igarashi (1984) and Häuser *et al.* (1993).

216- **Surface:** 0 = smooth (or nearly so), 1 = glandular or reticulate.

Häuser *et al.* (1993).

l) Larva:

217- **Osmeterium:** 0 = absent, 1 = present.

Ford (1944), Miller (character 1), De Jong *et al.* (1996, character 63), and Ackery *et al.* (1999). Present in Papilionidae and a characteristic of the group.

218- **Mature larva "neck":** 0 = absent, 1 = present.

De Jong *et al.* (1996, character 62) and Ackery *et al.* (1999). Present only in most Hesperiidae.

219- **Larvae type:** 0 = short and thick, 1 = cylindrical, 2= with humped thorax.

Ford (1944). Larvae are short and thick in Zerynthiini and cylindrical in

Parnassiini. Igarashi (1984) used "humped vs. cylindrical thoracic form".

220- Fleshy segmental tubercles: 0 = absent, 1 = present.

Ford (1944) and Munroe (1961). Fleshy tubercles are present in *Sericinus* and *Zerynthia*, etc. and absent in *Luehdorfia, Parnassius, Hypermnestra, Archon*, etc.

221- 1st instar larvae, bifid setae: 0 = absent, 1 = present on the head, 2 = present on thoracic and abdominal segments, 3=present on head, thorax and abdomen.

Miller (1987, characters 50 and 66).

222- 5th instar larval saddle mark: 0 = absent, 1 = present.

Igarashi (1984).

223- 5th instar larval head setae and tubercles: 0 = primitive, 1 = advanced.

Igarashi (1984). He defines this character as follows:

Primitive: with least numbers of primary setae; *Semi-advanced*: with more setae or dots added to the primitive pattern, *Advanced*: with numerous setae from which the counterparts of the setae of the primitive type cannot be distinguished. There were no species in this study with the intermediate condition, so it was omitted.

224- 5th instar larval head with two parallel downwards curved horns: 0 = absent, 1= present.

Le Cerf (1913); also noted by Häuser (1993). Unique to *Hypermnestra*.

225- 5th instar larval body setae and tubercles: 0=absent; smooth surface, 1= with setae, no tubercles; 2= with tubercles, no setae, 3= tubercles with setae.

Igarashi (1984) and Miller (1987, characters 86 and 103).

226- Resting habit of the larva: 0= underside of the leaf, 1= upper side of the leaf, 2= other.

Igarashi (1984) and Tyler *et al.,* 1994. Re-examination using additional literature identified some errors in the table provided by Tyler *et al.* (1994), including resting of larvae in *Hypermnestra*, *Archon*, *Luehdorfia,* etc. Examples of the third state include *Hylephila phyleus,* whose larvae stay concealed in silken tubes (Tashiro and Mitchell, 1985), *Plebejus acmon* whose larvae are myrmecophilous (Agrawal and Fortyce, 2000), and *Coenonympha tullia* where the larvae rest mostly on the trunk of the food plant.

m) Pupa:

227- Pupal shape: 0 = slender, 1 = stout.

Ford (1944), Igarashi (1984) and Carbonell (1996). Pupa is slender in *Zerynthia, Bhutanitis, Sericinus*, etc., and is stout in *Luehdorfia* and other Papilionidae.

228- Lateral ridges of pupa extending from cremaster to head: 0 = absent, 1 = present.

Miller (1987, character 51). Present in Graphiini.

229- Pupa with dorsal horns: 0 = absent, 1 = present.

Miller (1987, character 69). Present in *Graphium* only.

230- Mode of pupation: 0 = pupa enclosed in a cocoon in or on the ground, 1= suspended by girdle, cremaster, no cocoons, 2 = head and tail supporting type.

Ford (1944), Ehrlich (1958), Munroe (1961), Igarashi (1984), De Jong *et al.* (1996, character 103), and Stekolnikov and Kuznetsov (2003, character 6). Pupa is usually enclosed in a cocoon and is formed on or under the ground in *Parnassius, Hypermnestra* and *Archon*, while the cocoon is absent and the pupa it is suspended by girdle in Zerynthiini, as well as most Papilionidae and Pieridae. Girdle is usually absent in *Zerynthia* s.l. (Ford, 1944).

231- Metathoracic wing case in pupae: 0 = extending at least to abdominal segment 3; 1 = extending no further than abdominal segment 2.

Ackery *et al.* (1999) (for Lycaenidae).

o) Other:

232- **Chromosome number:** 0 = 30±1; 1 = 20-30; 2 = highly variable.

New character, coded mostly using Emmel *et al.* (1995).

233- **Anthoxanthin pigments:** 0 = absent or mostly absent, 1= present or mostly present.

Ford (1941; 1944a) and Hancock (1983).

234- **Red Pigments:** 0 = Type A, 1 = Type B, 2 = other.

Ford (1944, 1944a).

235- **Pterobilins:** 0 = absent, 1 = present.

Miller (1987, character 92). Present in *Graphium* clade.

236- **Pterin pigments in wing scales:** 0 = absent, 1 = present.

Ackery *et al.* (1999). Present in Pieridae.

Appendix 2. Characters excluded from this study.

1- **Second palpal segment, including investiture:** 0 = narrow, 1 = thicker, noticeably wider than third segment, 2 = very wide, very much thicker than third segment. De Jong *et al.* (1996, character 77).

Character states seemed arbitrary and could not be accurately coded.

2- **Length of forewing:** 0 = small, 1 = Large.

Ehrlich (1958) and Hancock (1983). Arbitrary measurement.

3- **Type of androconial scales:** Miller (1987) meticulously studies all the various types of androconial scales, present mostly on the wings of butterflies other than Parnassiinae. Beside "anal brushes" (character 67 above) uniformly present in all Papilioninae, various types of specialized scent scales can also be observed on the wings of Troinini and Graphiini (see Miller, characters 49, 57, 58, 91, 105, 143, 157, and 166). This diversity would be of great importance and should be included in any study on the phylogeny and evolution of subfamily Papilioninae, but they were excluded here simply because androconial scales are invariable in subfamily Parnassiinae.

4- **Type of female genitalia:** 0 = type A, 1 = type B, 2 = Type C.

Carbonell (1996, for *Allancastria*), and Saigusa and Lee (1982, for *Bhutanitis*). Type A is characterized by "ostium bursae" located on an extension of the sterigma in the shape of a more or less marked "strip" varying in species (from the most elongate to the shortest: *cerisyi, caucasica, cretica*) and the copulator channel developed (longest to shortest: *cerisyi, caucasica, cretica*). Type B is characterized by vaginal orifice which is not off-set on an out-growth of an abdominal plate and the copulator channel almost is non-existent (*louristana, deyrollei*). Type C is characterized by a U-shaed opening of the vaginal orifice (see Saigusa and Lee 1982, fig. 7-B2).

Other characters used in this study pertaining to genitalia already mostly duplicate these differences.

5- **Coloring of pupae:** 0 = brown only, 1 = alternating between green and brown.

Igarashi (1984). This character is phenotypically plastic in Papilionidae (Hazel, 1995).

6- **Average humidity of the habitat:** 0 = low (deserts) to moderate, 1 = moderate to high, 2 = high.

Often noted as an important ecological factor (e.g. De Freina and Naderi, 2003). Excluded due to difficulties associated with finding accurate data.

7- **Generations per year:** 0 = univoltine, 1 = multivoltine. Igarashi 1984.

Phylogenetically uninformative in the context of Parnassiinae, which includes only one multivoltine species (*Sericinus montela*). The rest are univoltine.

8- **Flight type:** 0 = slow, linear, low; 1 = slow, linear, medium to high; 2 = vigorous, fluttering, high; 3 = rapid, skipping, medium to high.

Ehrlich (1958) and Igarashi (1984). Excluded due to coding inaccuracy problems as some species may exhibit two or more categories.

9- **Wing pattern in male and female:** 0 = very similar, 1 = somewhat dissimilar, 2 = clearly dissimilar.

Hancock (1983). Excluded due to arbitrary and inaccurate assumptions. In some species (e.g. *Sericinus montela, Hypermenstra helios,* etc.) females can be either similar or dissimilar to males.

Appendix 3. Characters mapped on the phylogeny (not used to generate phylogeny):

1- **Distribution:** Eastern vs. western palaearctic, etc.

2- **Larval food plant:** Aristolochiaceae vs. non-Aristolochiaceae (Malvaceae, Poaceae, Polygonaceae, Brassicaceae, Fabaceae, Rosaceae, Annonaceae, Piperaceae, Umbelliferae, Rutaceae, Zygophyllaceae, Crassulaceae and Papaveraceae). Munroe (1961). *Aristolochia* vs. *Asarum* is also noted for Luehdorfiini (Hancock, 1983).

3- **Larvae gregariousness:** Gregarious vs. solitary. Igarashi (1984).

4- **Habitat type:** Open woodland, deserts, plains, forests, or alpine. Character coded mostly using Ford (1944).

Appendix 4. Specimens examined for morphological character coding.

Male and female specimens are shown as M and F. Numbers indicate University of Alberta Strickland Museum (UASM) specimen numbers, and are searchable (with UASM acronym followed by the five- or seven-digit number) at http://www.entomology.ualberta.ca/searching_fastfind.php. Seven-digit numbers starting with 990 indicate that the specimen is a DNA voucher; images and additional information for DNA vouchers can be viewed at http://www.biology.ualberta.ca/old_site/uasm//Vouchers/index.html. All specimens are deposited at the University of Alberta Strickland Museum.

Beside these specimens listed below, I have also used images from the following books for coding characters, particularly characters for wing pattern and genitalia: Bryk (1934, 1935), Tyler *et al.* (1994), Miller (1987), Weiss (1995-2005), Bascombe *et al.* (1999), Tschikolovets (1998-2003), and Nazari (2003).

Pyrgus communis: 9900901 (F; USA: CA), 24285 (M; Canada: AB), 18974 (F; Canada: AB). ***Hylephila phyleus***: 9900989 (M; USA: CA). ***Coenonympha tullia***: 9900984 (F; USA: CA), 74198 (M; Canada: AB), 74199 (F; Canada: AB). ***Plebejus acmon***: 9900969 (F; USA: CA), 21362 (M; Canada: AB), 21364 (F; Canada: AB). ***Colias eurytheme***: 9900543 (M; Canada: ON), 74200 (USA: AZ), 74201 (F; USA: AR), 74202 (F; USA: CA), 74230 (M; USA: CA). ***Pieris napi***: 9900943 (M; USA: CA), 74231 (M; USA: CA). ***Eurytides marcellus***: 74204 (M; USA: NK), 74205 (M; USA: NK). ***Graphium agamemnon***: 9900900 (M; SE Asia). ***Iphiclides podalirius***: 9900006 (M; France), 74234 (M; Hungary:

Budapest). ***Battus philenor***: 74203 (F; USA: SD), 74232 (M; USA: VA), 74244 (F; USA: FL). ***Parides photinus***: 9900149 (Costa Rica: Villa Colon), 74236 (M; El Salvador?), 74241 (M; Costa Rica: Villa Colon). ***Troides helena***: 9900974 (M; Malaysia), 74235 (M; Papua New Guinea). ***Papilio demoleus***: 74237 (M; Malaysia: Penang). ***Papilio machaon***: 74240 (M; USA). ***Papilio thoas***: 9900302 (F; French Guiana: Pointe Macouria), 74239 (M; Brazil: Companias). ***Baronia brevicornis***: 9900167 (F; Mexico: Teacalco), 9900938 (M; Mexico: Teacalco), 74233 (M; Mexico: Teacalco). ***Hypermnestra helios***: 9902026 (M; Turkmenistan: Kara Kala), 9902067 (M; Iran: Tehran), 9902068 (M; Iran: Hormozgan), 9902069 (F; Uzbekistan: Fergana Valley), 9902070 (M; Tajikstan: S. Dzhilikul), 9902071 (F; Kazakhstan: Bakanas Village), 74229 (M; Uzbekistan: Fergana Valley), 74227 (M; Turkmenistan: Kara-Kala). ***Parnassius phoebus***: 9900008 (M; Canada: AB), 74242 (Male; Canada: AB). ***Parnassius tenedius***: 9901784 (M; Kirgizstan: Aktash Village). ***Parnassius delphius***: 9901775 (M; Kirgizstan: Naryntoo Mts.). *Parnassius simonius* (Staudinger, 1889): 9901777 (M; Kirgizstan: Transalai Mts.). ***Parnassius clodius***: 9900375 (F; USA: WA), 74243 (Male; USA: CA). ***Archon apollinaris apollinaris***: 9902025 (M; Iran; Kermanshah), 9902060 (M; Turkey: Mardin-Diyarbakir), 9902061 (M; Iran: W. Azerbaijan), 9902062 (Male: Iran, Lorestan), 9902065 (M; Iran: Kermanshah), 74228 (M; Iran: W. Azerbaijan). ***Archon apollinaris bostanchii***: 9902063 (M; Iran: Lorestan), 9902064 (F; Iran: Lorestan). ***Archon apollinus***: 9902024 (M; Israel: Emaus), 9902059 (F; Turkey: Yaliciftilic), 74226 (M; Turkey: Yaliciftilic). ***Luehdorfia chinensis***: 9902031 (M; China: Lishui), 9902091 (F; China:

Jiangshu), 9902103 (M; China: Jiangshu), 9902104 (M; China: Zhejiang). ***Luehdorfia japonica***: 9900335 (M; Japan: Kanazawa), 9902033 (F; Japan: Niigata), 74222 (M; Japan: Niigata). ***Luehdorfia puziloi***: 9902030 (F; China: Liaoning), 9902093 (M; Korea: near Seoul), 9902109 (M; China, Liaoning), 9902110 (F; Korea: near Seoul), 74220 (M; Russia: Slavianca), 74221 (M; Korea: near Seoul). ***Luehdorfia taibai***: 9902032 (M; China: Qinling), 9902092 (M; China), 9902102 (F; China: Shaanxi), 9902107, 9902108, 74223 (M; China: Shaanxi). ***Sercicinus montela***: 9900399 (M; Japan: near Tokyo), 9902027 (M; China: Jilin), 9902028 (M; Russia: Primoriye), 9902029 (F; China: Ningbo), 9902090 (F; Korea: near Seoul), 9902111 (F; China: Zhejiang), 74224 (M; Korea: near Seoul). ***Bhutanitis lidderdali***: 9902044 (M; China: Yunnan), 74225 (Male: China). ***Bhutanitis mansfieldi***: 9902041 (M; China: Sichuan), 9902042 (F; China: Yunnan), 9902097 (F; China: Yunnan). ***Bhutanitis thaidiana***: 9902043 (M; China: Sichuan). ***Zerynthia polyxena***: 9902045 (F; Montenegro), 9902066 (M; Greece; Florina), 9902072 (M; Kosovo), 9902073 (M; Serbia), 9902074 (F; S. Russia), 9902075 (M; SE Ukraine), 74217 (M; Serbia: Petlovo Brdo), 74218 (M; Greece: Florina), 74219 (M; Serbia: Kosovo). ***Zerynthia rumina***: 9900088 (M; Spain: Malaga), 9902039 (F; Spain: Catalonia), 9902040 (M; Morocco: near Casablanca), 9902047 (F; Morocco: Agadir), 9902048 (M; France: Ardechne), 9902049 (M; Spain: Granada), 9902050 (Spain: Caceres). 9902051 (Male: Spain, Hervas), 9902052 (F; Spain: Pozazal), 9902053 (M; Spain: Islallana), 9902054Female, Spin: Nidaguila), 9902055 (M; Spain: Temino), 9902056 (M; Spain: Hortiguila), 9902057 (Female; Spain: Malaga), 9902058 (Male: Portugal; Algarve), 74210 (M; Spain: Islallana), 74211 (M; Spain: Nidaguila), 74212 (M; Spain: Temino), 74213 (M; Spain: Hortiguela), 74214 (M; Spain: Hervas), 74215 (M; Spain: Pozazal), 74216 (M; Spain: Granada). ***Allancastria caucasica***: 9902046 (M; Turkey; Bolu), 9902100 (M; Russia: W. Caucasus), 9902113 (F; Russia: W. Caucasus). ***Allancastria cerisyi***: 9900342 (Greece: Thessalaniki), 9902034 (M; Israel), 9902076 (M; Greece: Cyprus), 9902077 (M; Turkey; Davutlar), 9902078 (M; Greece: Florina), 9902079 (F; Macedonia), 9902080 (M; Macedonia), 9902081 (M; Kosovo), 9902082 (M; Macedonia-Bulgaria-Greece border), 74206 (M; Serbia: Kosovo), 74207 (M; Israel), 74238 (M; Greece: Thessalaniki). ***Allancastria cretica***: 9902038 (M; Greece: Crete). ***Allancastria louristana***: 9902037 (M; Iran: Lorestan), 9902101 (M; Iran, Lorestan), 9902114 (M; Iran: Lorestan), 74208 (M; Iran: Lorestan). ***Allancastria deyrollei***: 9902035 (F; Israel), 9902036 (M; Iran: Kermanshah), 9902083 (M; Israel), 9902084 (M; Turkey: Nigde), 9902085 (M; Turkey: Ankara), 9902086 (M; Iran: W Azerbaijan), 9902088 (F; Turkey: Van), 74209 (M; Iran; Kermanshah).

Appendix 5. Coding for 236 morphological characters used in this study. Charater states are 0-5, ? (indeterminate), and - (inapplicable). Refer to Appendix 1 for character descriptions.

	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	5555555556 1234567890	6666666667 1234567890	7777777778 1234567890	8888888889 1234567890	1 9999999990 1234567890
Colias eurytheme	1100400000	0010103-21	0002001100	0001101002	0211012021	0000000011	1001010000	1100301101	200010--00	0100-11101
Pieris napi	1100400000	0010103-21	0002001101	0001101002	0211010111	0000000011	1001010000	0000201101	2000011000	0100-21101
Plebejus acmon	1100000311	001?103-21	2102101111	000?011100	0131000110	1000000001	0003010001	-000201101	000100--00	0100-01110
Coenonympha tullia	1100301110	001?002111	2102001110	0100111110	0021000120	1100000001	0001010000	1121301101	0000011000	0100-11101
Pyrgus communis	0101000?00	211?001000	0001010000	0????01000	1011000100	1011001100	0101010011	1001011101	000100--01	1100-01110
Hylephila phyleus	0101000?00	211?001000	1001010000	0????01000	1111000110	1001001100	0101010011	1001011-01	020100--01	1100-00-10
Iphiclides podalirius	1100100200	1000002?21	0012001100	1110001002	1211102021	1000010?0?	???0001100	0011111012	0110011000	0000101000
Graphium agamemnon	1100100200	1000002-21	0012001100	1110001002	1211102021	1000010001	0110011100	0011110012	0010011000	0000101100
Eurytides marcellus	1100100200	1000002?21	0012001100	1110001002	1211102021	1000010?0?	???0011100	0011111012	0010011000	0000101000
Battus philenor	1110200100	1000002?21	0012001101	1111100002	1211102021	1000010?0?	???0111100	0011211012	1000011000	0011101000
Troides helena	1110200100	1000002-21	0012001101	1111100002	1211102021	1000010001	0111111100	0011211012	1100011000	0010101001
Parides photinus	1110200100	1000002?21	0012001101	1111100002	1201102021	1000010?0?	???0111100	0011211012	0000011000	0010001000
Papilio thoas	1110100100	1001002?21	0012001101	1111100002	1211102021	1000010?0?	???1101000	0011111012	0000011000	0000101100
Papilio machaon	1110100100	1001002-21	0012001101	1111100002	1211102021	1000010001	0110101000	0011111012	0000012000	0000101100
Papilio demoleus	1110100100	1001002?21	0012001101	1111100002	1211102021	1000010?0?	???1101000	0011111012	0000011000	0000101100
Baronia brevicornis	1011100100	1000002121	0002001100	1000001002	1211001021	0000010001	0110000000	0010210001	0110010110	0100-01001
Hypermnestra helios	1001?00100	1010012?11	0012001100	11?0001002	1211001021	0000210?0?	???0000000	1010311001	0100011000	0000101000
Parnassius phoebus	1001100100	1010012011	0012001100	1100001002	2201102021	1000010001	0114000000	1010211001	0200010100	0000101000
Parnassius hardwickii	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000010001	0114000000	101021100?	0200010100	0000101000
Parnassius schultei	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000010001	0114000000	1010211001	0200010100	0000101000
Parnassius tenedius	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000010001	0114000000	1010221001	0200010100	0000101000
Parnassius delphius	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000010001	0114000000	1010211001	0200010100	0000101000
Parnassius autocrator	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000110001	0114000000	1010201002	0200010100	0000101000
Parnassius simonius	1001?00100	1010012?11	0012001100	1100001002	1201102021	1000110001	0114000000	1010221002	0200010100	0000101000
Parnassius clodius	1001?00100	1010012?11	0012001100	1100001002	2201102021	1000110001	0114000000	1010211000	0200010100	0000101000
Archon apollinaris	1100?00100	1000002?11	0012001100	1100001001	1201102021	1000010001	0112000000	1011201001	0100011100	0000001001
Archon apollinus	1100?00100	1000002?11	0012001100	1100001001	1201102021	1000010001	0112000000	1011201001	0100011100	0000001001
Archon bostanchii	1100?00100	1000002?11	0012001100	1100001001	120?10?0??	????010001	0112000000	1011201001	0100011100	0000001001
Luehdorfia japonica	1110?00100	1010002?11	0012001100	1100000001	1101102021	1000010001	0110000000	1011201001	0100010100	0000101000
Luehdorfia puziloi	1110?00100	1010002?11	0012001100	1100000002	1201102021	1000010001	0110000000	1011201001	0100010100	0000101000
Luehdorfia chinensis	1110?00100	1010002?11	0012001100	110000000?	1?0??0?0??	????010001	0110000000	1011201001	0100010100	0000101000
Luehdorfia taibai	1110?00100	1010002?11	0012001100	1100000001	1101102021	1000010001	0110000000	1011201001	0100010100	0000101000
Sericinus montela	1010?00100	1010002?11	0012001101	1100000002	1201102021	1000010001	0110000000	1011111001	0100011010	0000101101
Bhutanitis thaidiana	1010?00100	1010002?11	0012001101	1100000001	110?102021	????010001	0111000000	1011201001	0300011110	0000001001
Bhutanitis mansfieldi	1110?10110	1010002?11	0012001101	1100000001	1101102021	1000010001	0110000000	1011201001	0300011110	0000001001
Bhutanitis lidderdalii	1010100100	1010002011	0012001101	1100000001	1101102021	0000010001	0111000000	1011201001	0300011110	0000101001
Bhutanitis ludlowi	1010?00100	1010002?11	0012001101	110000000?	1?0??0?0??	????010001	011?000000	1011201001	0300011110	0000001001
Zerynthia rumina	1110?00100	1010002?11	0012001101	1100000002	1101102021	1000010001	0110000000	1011201001	0100011100	0000001000
Zerynthia polyxena	1110?00100	1010002?11	0012001101	1100000002	1201102021	1000010001	0110000000	1011201001	0100011100	0000001000
Allancastria louristana	1110?00100	1010002?11	0012001101	1100000002	1101102021	1000010001	0110000000	1011201001	0200011100	0000001000
Allancastria deyrollei	1110?00100	1010002?11	0012001101	1100000002	1201102021	1000010001	0110000000	1011201001	0200011100	0000001000
Allancastria cretica	1110?00100	1010002?11	0012001101	1100000002	120?102021	????010001	0110000000	1011201001	0200011100	0000001000
Allancastria cerisyi	1110?00100	1010002?11	0012001101	1100000001	1101102021	1000010001	0110000000	1011201001	0200011100	0000001000
Allancastria caucasica	1110?00100	1010002?11	0012001101	110000000?	1?0??0?0??	????010001	0110000000	1011201001	0200011100	0000001000
Thaites ruminiana	10?0200?00	1010?0????	?????????1	??????????	??????????	??????????	?????0?020	10?1221001	01000111?0	0000101101
Doritites bosniackii	1?????0?00	10???0????	??????????	?????01002	?201102021	1000??????	?????0?000	1011201001	0100011100	0?00?01101
Prepapilio colorado	1?????0?00	101??0????	?????0???1	1?????????	??????????	????0?????	?????0?000	0010301112	0020011100	0100-21110

	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111111	1111111112
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	9999999990
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
Colias eurytheme	00-00000??	??????????	??????????	??????????	??????000?	??????????	1100000010	120???0011	1000001200	0301??????
Pieris napi	00-00000??	??????????	??????????	??????????	??????000?	??????????	0100000010	1200--0000	0000000101	02001?????
Plebeius acmon	00--0000??	??????????	??????????	??????????	??????000?	??????????	0100011110	0201110020	1100000100	10010?????
Coenonympha tullia	00-00000??	??????????	??????????	??????????	??????000?	??????????	?100000120	0200--1010	0000000100	1201??????
Pyrgus communis	00--0000??	??????????	??????????	??????????	??????000?	??????????	?100010010	120???0021	1100000010	10210?????
Hylephila phyleus	00--0000??	??????????	??????????	??????????	??????000?	??????????	1100010010	1300--0021	1100020100	10210?????
Iphiclides podalirius	0101001001	010-100000	0001000011	1000100000	0000001111	1011001121	0040011000	040???0020	0011220010	021121??11
Graphium agamemnon	0101001001	0110100010	0001000010	1010100100	1000000110	0001010100	1040011000	040???0000	0021222010	120101?011
Eurytides marcellus	0101001001	0110000010	0011000011	1010100000	1000001111	1111001121	1000020011	010???0000	0020221101	021111?011
Battus philenor	010000001-	----------	--------0-	----------	-0000-0111	11111110-1	2040011000	040???0021	0010122001	020101?011
Troides helena	00-000111-	----------	---0001000	----------	-0000-0000	00000100-1	2040011001	040???0000	1010120101	000001?011
Parides photinus	010010111-	----------	-----01100	----------	-0000-0111	1111010130	2040011010	120???0011	0020121101	020101?011
Papilio thoas	0100001001	0010100010	0011100011	0000000?00	-0000-0000	1101011101	2040010000	310???0010	0020120001	000011?011
Papilio machaon	0100001001	0010100010	0111100011	0010100100	00000-0000	1101111101	2040011000	040???0020	0010122001	020011?011
Papilio demoleus	00-0001001	0010100010	0111100011	00101?0000	1000000111	1111101101	2040011000	040???0020	0010120101	020011?011
Baronia brevicornis	00-0000000	0010100010	0000000010	0010000-00	0000000111	1111010100	0100000010	1200??0121	0000000101	000110?11?
Hypermnestra helios	00-0000000	010-111100	0100000010	0000011002	0111100101	0011010100	0100000010	120???0000	0010020101	0201110011
Parnassius phoebus	00-0000000	010-111100	0000100010	0000011100	11111-0101	11110000-0	3100010010	121???1010	0010010210	0211111010
Parnassius hardwickii	00-0000000	010-111100	0000110012	0000011100	11111-1101	11110010-0	0100010010	120???0000	0010010210	0011111010
Parnassius schultei	00-0000000	010-101000	0000100011	0000011100	10000-0000	00000000-0	0100010010	120???0000	0010010210	0011111010
Parnassius tenedius	00-0000000	010-111000	0000110010	0000011100	10111-0101	11110000-0	0100010020	100???1010	0010010210	1??1?11010
Parnassius delphius	00-0000000	010-101000	0000100011	0000011100	10110-0101	00110000-0	0100010000	120???0020	0010010210	10?1011010
Parnassius autocrator	00-0000000	010-100000	0000101011	0000011100	10000-0101	11110010-0	0100010010	120???0000	0010010210	1001011010
Parnassius simonius	00-0000000	010-101000	0000100010	0000011100	10010-0101	00000000-0	0100010000	120???0021	0010010210	1301011010
Parnassius clodius	00-0000000	010-101100	0000100010	0000011100	10111-0101	00110000-0	0100010110	130???0021	0010010210	1001011010
Archon apollinaris	00-0000000	010-010100	0000101101	0000010100	00000-0111	11111-00-0	0100010010	220???1020	0000012000	1011011010
Archon apollinus	00-0000000	010-010100	0000101101	0000010100	00000-0111	11111-00-0	0100010010	120???1020	0000012000	1011011010
Archon bostanchii	00-0000000	010-010100	0000101101	0000010100	00000-0111	11111-00-0	0100010010	120???1020	0000012000	1211011010
Luehdorfia japonica	0100002001	0110101010	1001101011	1010100001	1000000001	1111111110	0130010020	1002100001	0001023110	101101?01-
Luehdorfia puziloi	0100002001	0110101010	1001101011	1010100001	1000000001	1111111110	0130010020	1002100011	0002023210	101101?00-
Luehdorfia chinensis	0100002001	0110101010	1001101011	1010100001	1000000001	1111111110	0130110020	1002110010	0001023110	101101?00-
Luehdorfia taibai	1100002001	0110101010	1001101011	1010100001	1000000001	1111111110	0130010020	1002100011	0002023110	101101?00-
Sericinus montela	0100001000	0110111111	0001001011	1010111102	0000010111	1111111101	0121-10000	1101100021	1110022010	121111?001
Bhutanitis thaidiana	0110112001	0110101010	1001101011	1010100012	0000010001	1111101111	0100-10020	11010-0011	1000021200	1001110011
Bhutanitis mansfieldi	0110112001	0110101010	1001101011	1010100012	0000010001	1111101111	0121-10020	11010-0021	0000021200	1001110011
Bhutanitis lidderdalii	1100112001	0110101010	1001101011	1010100002	0000010001	1111101110	0111-10020	11010-0021	0000021200	1002110011
Bhutanitis ludlowi	0110112001	0110101010	1001101011	1010100002	0000010001	1111101111	??????????	??????????	??????????	??????????
Zerynthia rumina	00-0001001	1111111100	0101111011	1111111102	0111110101	1111111111	0100010000	1301??0021	0110020200	0101?12011
Zerynthia polyxena	00-0001001	0110111000	0101101011	1111110102	0001110101	1111111111	0100010000	1301??0021	0110020200	0011?12011
Allancastria louristana	00-0001001	0110101001	1001101010	1010000000	0000010101	1111010101	0100010000	120???0020	0000020100	0011012011
Allancastria deyrollei	0100111001	0110101001	1001101010	1010000000	0000010101	1111010101	0100010010	120???0020	0000020100	0011012011
Allancastria cretica	0100111001	0110101001	1001101011	1010000000	0000010101	1111010101	0100010010	120???0021	0000020100	0011012011
Allancastria cerisyi	0100111001	0110101001	1001101011	1010000000	0000010101	1111010101	0100010010	120???0021	0000020100	1011012011
Allancastria caucasica	0100001001	0110101001	1010101011	1010000000	0000010101	1111010101	0100010010	120???0021	0000020100	1011012011
Thaites ruminiana	00-0000001	010-001010	00?0100?1?	0?0?1??100	?????-????	????11?1?0	??????????	??????????	??????????	??????????
Doritites bosniackii	00-0000001	000-000000	000110-000	1?0?0??101	?????0????	????-100-0	??????????	??????????	??????????	??????????
Prepapilio colorado	0100000001	0010100010	0001101111	??????????	?????-1111	1111?11100	??????????	??????????	??????????	??????????

	2222222222 0000000001 1234567890	2222222222 1111111112 1234567890	2222222222 2222222223 1234567890	222222 333333 123456
Colias eurytheme	??0???0???	0?30310010	0070110001	00??01
Pieris napi	??0???0???	0130310010	0010110001	01??01
Plebeius acmon	??0???????	0???110000	00?0021001	11??00
Coenonympha tullia	??1???????	0???000010	0000021000	00??00
Pyrgus communis	??0???????	0???010110	0000111000	00??00
Hylephila phyleus	??0???????	0???000110	2000020000	00??00
Iphiclides podalirius	0?0???1??2	0120001020	3010011101	0?0200
Graphium agamemnon	0?0???1??2	0250001020	2000011111	001010
Eurytides marcellus	0?0???0??2	0120001020	20?0011101	020000
Battus philenor	0?0???1???	0040011011	00?0211001	000100
Troides helena	0?0???1??1	0010001011	0100201001	000000
Parides photinus	0?0???1???	1031011011	01?0211001	000000
Papilio thoas	0?0???1??2	0140001020	0110011001	000100
Papilio machaon	0?0???1??2	0140001020	0110011001	000100
Papilio demoleus	0?0???1??2	0140001020	0110011001	000100
Baronia brevicornis	-?0???1??0	0230101010	101000100?	0?0000
Hypermnestra helios	-?0???0??2	0???001010	0001111000	020000
Parnassius phoebus	-?0???1??2	100-211010	0000111000	001000
Parnassius hardwickii	-?0???1??2	100-001010	0000111000	001000
Parnassius schultei	-?0???1??2	100-??1010	0000111000	001000
Parnassius tenedius	-?0???1??2	100-??1010	0000111000	001000
Parnassius delphius	-?0???1??2	100-201010	0000111000	001000
Parnassius autocrator	-?0???1??2	100-201010	0000111000	001000
Parnassius simonius	-?0???1??2	100-201010	0000111000	001000
Parnassius clodius	-?0???1??2	100-211010	0000111000	001000
Archon apollinaris	-?0???1??2	010-001010	0000121000	000000
Archon apollinus	-?0???1??2	010-001010	0000121000	000000
Archon bostanchii	-?0???1??2	010-001010	0000121000	000000
Luehdorfia japonica	-?00011??2	110-001000	0000101001	000000
Luehdorfia puziloi	-?00001?12	110-001000	0000101001	000000
Luehdorfia chinensis	-?000?1??2	120-001000	0000101001	000000
Luehdorfia taibai	-?00001?02	110-001000	0000101001	000000
Sericinus montela	110???1??2	0???001001	0000300001	000000
Bhutanitis thaidiana	11011?12?2	100-001001	0000300001	0?0000
Bhutanitis mansfieldi	11011?11?2	100-001001	0000300001	0?0000
Bhutanitis lidderdalii	11011?10?2	100-001001	0000300001	0?0000
Bhutanitis ludlowi	??????????	????001001	0000300001	0?0000
Zerynthia rumina	100???1??2	000-001001	0000310002	000000
Zerynthia polyxena	100???1??2	000-001001	0000310002	000000
Allancastria louristana	100???1??2	00??001001	0000310002	000000
Allancastria deyrollei	100???1??2	00??001001	0000310002	000000
Allancastria cretica	100???1??2	00??001001	0000310002	000000
Allancastria cerisyi	100???1??2	00??001001	0000310002	000000
Allancastria caucasica	100???1??2	00??001001	0000310002	000000
Thaites ruminiana	??????????	??????????	??????????	??????
Doritites bosniackii	??????????	1?????????	??????????	??????
Prepapilio colorado	??????????	1?????????	??????????	??????

Appendix 6. Primers used in chapter 2.

Gene	Location	Primer	Source	F/R[*]	Sequence (5'→3')
COI [a]	1460	K698	Sperling *et al.* 1994	F	TAC AAT TTA TCG CCT AAA CTT CAG CC
	1840	K699	Sperling et *al.* 1995	R	AGG AGG ATA AAC AGT TCA YCC
	1751	RonIII	Caterino & Sperling 1999	F	GGA GCA CCT GAC ATA GCT TTC CC
	2183	Jerry	Simon et *al.* 1994	F	CAA CAT TTA TTT TGA TTT TTT GG
	2329	K525	Simon et *al.* 1994	R	ACT GTA AAT ATA TGA TGA GCT CA
	2329	K525.2	Caterino et *al.* 2001	R	ACA GTA AAT ATA TGA TGA GCT CA
	2329	K525.4	Caterino et *al.* 2001	R	ACT GTG AAT ATG TGA TGG GCT CA
	2495	BrianXXI	Caterino et *al.* 2001	F	CCT CAA TTT TAT GAA GAT TAG G
	2658	Mila7	Caterino & Sperling 1999	R	GAA AGT CCA GTA AAT AAA GG
	2837	George	Bogdanowicz *et al.* 1993	F	ATA CCT CGA CGT TAT TCA GA
tRNA Leu[a]	3014	Pat	Simon et *al.* 1994	R	TCC AAT GCA CTA ATC TGC CAT ATT A
	3014	PatII	Sperling et *al.* 1996	R	TCC ATT ACA TAT AAT CTG CCA TAT TAG
	3038	Patrick	Caterino et al. 2001	F	CTA ATA TGG CAG ATT ATA TGT AAT GGA
COII [a]	3138	PierreIII	new	F	AGA GTT TCA CCT TTA ATA GAA CA
	3389	Marilyn	Simon et *al.* 1994	R	TCA TAA GTT CAR TAT CAT TG
	3389	MarilynII	Caterino & Sperling 1999	R	TCA TAW CTT CAR TAT CAT TG
	3782	Eva	Bogdanowicz *et al.* 1993	R	GAG ACC ATT ACT TGC TTT CAG TCA TCT
ND5 [a]	6656	A1	Yagi et *al.* 1999	R	AAT ATD AGG TAT AAA TCA TAT
	7080	C2	Yagi et *al.* 1999	R	ATC YTT WGA ATA AAA YCC AGC
	7095	A3	Yagi et *al.* 1999	F	TTC GAA TTT AGC TTT ATG TGG
	7490	V1	Yagi et *al.* 1999	F	CCT GTT TCT GCT TAA GTT CA
ND1 [a]	12075	[unnamed]	Aubert et *al.* 1999	F	ATC AAA AGG AGC TCG ATT AGT TTC
	12567	[unnamed]	Aubert et *al.* 1999	R	CGT AAA GTC CTA GGT TAT ATT CAG ATT CG
	12585	ND1A	Simon et *al.* 1994	F	GGT CCC TTA CGA ATT TGA ATA TAT CCT
	12313	Faw ND1A (K595)	Simon et *al.* 1994	R	TAG AAT TAG AAG ATC AAC CAG
16S [a]	12887	(nr LR-J-12887)	Aubert et *al.* 1999	F	CCG GTT TGA GCT CAG ATC
	13398	(nr LR-N-13398)	Aubert et *al.* 1999	R	CGC CTG TTT ATC AAA AAC AT
EF1a [b]	0	Starsky	Cho et *al.* 1995	F	CAC ATY AAC ATT GTC GTS ATY GG
	15	Papsky	Reed & Sperling 1999	F	CGG ACA CGT CGA CTC CGG
	174	Bo	Cho et *al.* 1995	F	GCT GAG CGY GAR CGT GGT ATC AC
	238	Hutch	Cho et *al.* 1995	R	CTT GAT GAA ATC YCT GTG TCC
	479	Laverne	Cho et *al.* 1995	F	GAG GAA ATY AAR AAG GAA G
	541	Luke	Cho et *al.* 1995	R	CAT RTT GTC KCC GTG CCA KCC
	551	Petra	Caterino et *al.* 2001	R	TGG CTC CAG CAT GTT GTC TCC
	729	BJ	Cho et *al.* 1995	F	CAR GAC GTA TAC AAA ATC GG
	746	Verdi3	new	R	GAC ACC AGT TTC AAC TCT GCC
	927	Buck	Cho et *al.* 1995	F	CGT CAA GGA RYT GCG TCG TGG
	1048	Bear	Cho et *al.* 1995	R	GCA ATG TGR GCI GTG TGG CA
	1241	Tweeky	Cho et *al.* 1995	R	ACA GCV ACK GTY TGY CTC ATR
wg [c]	275	Lepwg-1	Brower & Desalle 1998	F	GAR TGY AAR TGY CAY GGY ATG TCT GG
	679	modLepwg-2	Brower & Desalle 1998	R	ACT ICG CAR CAC CAR TGG AAT GTR CA

[*] **Forward or reverse direction**

[a] **Positions relative to *Drosophila yakuba* mtDNA (Clary and Wolstenholme, 1985).**

[b] **Positions relative to *Heliothodes diminutivus* (Cho et al., 1995).**

[c] **Positions relative to *Juninia coenia* (Carroll et al., 1994)**

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Autobiographical Sketch

I was born September 4th, 1974 in Tehran, Iran, to Parkouhi (Helen) Soleymani and Manavaz Nazari, of Armenian descent. I have a sister and a brother, both younger than myself. I grew up and finished my elementary and high schools in Tehran.

My interest in butterflies first showed itself at the age of 11, when one afternoon I accidentally hit an *Iphiclides podalirius* while I was playing badminton with my cousin in our backyard. I was immediately hooked by the sense of a newly discovered beauty, and I put the unprofessionally mounted bug up on the wall of my room. The next day, I borrowed a book from the library on how to make a butterfly net and a collection.

By the age of 16, I had a fairly large collection of butterflies from Tehran and suburbs. My biology teacher in the high school, Mr. Ebrahimi, gave me the chance to put parts of my collection on demonstration. The event was noticed by people from the local education board, who later introduced me to the largest insect collection of Iran (and the entire region) in the Plant Pests and Diseases Research Institute (PPDRI) in Tehran.

A few years later, when I graduated high school in 1993, I was hired by PPDRI's Insect Taxonomy Research Department as a technician of Lepidoptera, and I worked there part time for over 3 years. I was mentored by such great entomologists as the late Hayk Mirzayans. During that period, beside the regular job of curating the Lepidoptera collection, I was actively involved in numerous expeditions all across Iran, collecting butterflies, moths, or other insects.

Realizing that there were no books on butterflies of Iran, I started writing my own around late 1993. I got my undergraduate degree early in 1998 from Azad University (Tehran) in English Translation. At that time I was working for the National Museum of Natural History in Tehran, again as a technician in the Invertebrate collection. The Museum is part of the Department of Environment, the director of which is the deputy prime minister of the country. I worked in this position for about a year. In 1999 I was offered a new position as secretary of the National Biodiversity Strategy and Action Plan, an international project funded by the United Nations and implemented by the Iranian Department of Environment. I accepted the offer and was on the job up until December 2002. During that period and on a break, I served my (obligatory) military service as an English teacher in the Iranian Navy during 1998-2000. It was during this period that I completed a major part of the text for my book. When it was finished later in 2001, I asked a good friend of mine, Bijan Farhang Darre-Shouri, a professional photographer of nature, to take the pictures of butterflies for my book. I spent the period between April to November 2002 in the publisher's graphics office, setting up the plates and maps for the book. Right before my departure to Canada in December 2002, I handed the signed, final draft to the publisher. The book was published a year later, and I received the first copy through mail.

In 2002 I met my supervisor, Dr Felix Sperling in the annual meeting of the Societas Lepidopterologica Europeae (SEL) held in Korsor, Denmark, and I had fruitful discussions with him regarding the possibility of pursuing a masters degree in Canada. Upon admission to University of Alberta at the same year, I moved to Edmonton in January 2003 with my wife, Meline Petrosians. We have lived here ever since, and our son, Norbert, was born in Edmonton on November 24, 2003.